Uncovering Biosynthetic Potential of Plant-Associated Fungi: Effect of Culture Conditions on Metabolite Production by *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii*[1]

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In an attempt to uncover the biosynthetic potential of plant-associated fungi, the effect of culture conditions on metabolite production by *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii* was investigated. These studies indicated that the production of the major metabolites by *P. quadriseptata* differ when the water used to make the media was changed from tap water to distilled water. It resulted in the isolation of six new secondary metabolites, cytosporones F−I (**1**−**4**), quadriseptin A (**5**), and 5′-hydroxymonocillin III (**6**) together with monocillin III (**7**), a metabolite new to *P. quadriseptata*, in addition to monocillin I (**8**), a previously known metabolite from this organism. Aposphaerin B (**9**) encountered was suspected to be an artifact originating from cytosporone F (**1**). Incorporation of heavy metal ions to *P. quadriseptata* culture medium induced production of monocillin I (**8**) by this fungus. Cultivation of *C. chiversii* in liquid medium resulted in the isolation of chaetochromin A (**12**) as the major metabolite instead of radicicol (**10**), the major constituent of this organism when grown in a solid medium. Compounds **1**−**7** and **12** were evaluated for their potential to inhibit Hsp90 and antiproliferative activity toward the cancer cell lines NCI-H460, MCF-7, and SF-268. Only compounds **6**, **7**, and **8** exhibited significant activity in both assays.

Plant-associated microorganisms represent a largely untapped resource of small-molecule natural products, some with chemical structures that have been optimized by coevolution for biological and ecological relevance.[2] According to Bode et al.,[3] with more than 20 000 compounds described in the literature, microorganisms must be called metabolic artists superior to any metabolic diversity created by man. This ability of microorganisms combined with the potential to bring up a variety of new metabolites from a single strain by systematic alteration of its cultivation parameters, known as OSMAC (one strain many compounds) approach,[4] and the use of elicitors to induce or inhibit certain biosynthetic and/or signal transduction pathways[5] provide new opportunities to maximize chemical diversity of their metabolites. The OSMAC approach has resulted from the observation that very small changes in the cultivation conditions can completely shift the metabolic profile of many microorganisms. Thus, it represents a powerful tool to elucidate the secondary metabolome (the overall number of all secondary metabolites of one organism) of different microbes. This approach has recently been used to release the chemical diversity of a number of soil-borne fungi and actinomycetes[6] and a fungus of marine origin.[7]

In a study to uncover the chemical diversity of plant-associated microorganisms, we have investigated the influence of culture conditions on metabolite production of the fungal strains *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii*. We have previously reported that the rhizosphere fungal strain *P. quadriseptata*, when cultivated in potato dextrose agar (PDA) and potato dextrose broth (PDB; a medium with a similar constitution to PDA but without any added agar) media made up in tap water, produced the C_{18} polyketide monocillin I (**8**) as the major metabolite,[8a] together with the minor isocoumarins paraphaeosphaerins A−C, biosynthetically related to **8**, aposphaerin C (**11**), eugenetin (**13**), 6-methoxymethyleugenin (**14**), and 6-hydroxymethyleugenin (**15**).[9] Similarly, the endophytic fungal strain *C. chiversii*, when cultivated in PDA medium made up in tap water, produced the corresponding metabolites radicicol (**10**)[8b] and chaetochiversins A and B.[9] In the present study, we have investigated the metabolite profiles of these organisms cultivated in PDB medium made up separately in tap water and distilled water, and herein we report the isolation of six new metabolites, cytosporones F−I (**1**−**4**), quadriseptin A (**5**), and 5′-hydroxymonocillin III (**6**), together with monocillin III (**7**), monocillin I (**8**), and aposphaerin B (**9**) from *P. quadriseptata* and the known metabolites chaetochromin A (**12**), eugenetin (**13**), and 6-methoxymethyleugenin (**14**) from *C. chiversii*. The octaketide metabolites structurally related to **1**−**4**, namely, cytosporones A−E and dothiorelone A, have previously been encountered in *Cytospora* sp. CR200 and *Diaporthe* sp. CR146,[10] and *Dothiorella* sp. HTF3,[11] respectively.

Results and Discussion

The rhizosphere fungus *P. quadriseptata*[8a] was cultivated for 14 days in PDB media made up separately with distilled water and tap water, the culture supernatants were extracted with EtOAc, and the resulting extracts were analyzed by HPLC. The HPLC profiles of the two extracts indicated that they contained almost the same metabolites but with significant differences in their major constituents (Figure 1). Thus, they were separately processed to isolate and characterize the major metabolites present in these extracts. Fractionation of the EtOAc extract of *P. quadriseptata* cultured in distilled water involving reversed-phase column chromatography and silica gel preparative TLC furnished cytosporones F (**1**) and G (**2**), quadriseptin A (**5**), monocillin I (**8**), and aposphaerin B (**9**). Cytosporone F (**1**) was determined to have the molecular formula $C_{18}H_{22}O_5$ by a combination of HRFABMS, ^{13}C NMR, and HSQC data and indicated eight degrees of unsaturation. The IR spectrum had absorption bands due to OH (3369 cm^{-1}), ester carbonyl (1732 cm^{-1}), and α,β-unsaturated ketone carbonyl (1724 cm^{-1}) groups. The ^1H NMR spectrum exhibited signals due to a chelated OH (δ 11.51), two aromatic protons at δ 6.31, a 2H singlet at δ 3.77 due to a CH_2 group sandwiched by an aromatic ring and an ester carbonyl, an $-OCH_2CH_3$ group [δ 4.16 (2H, q, J = 7.1 Hz) and 1.25 (3H, t, J = 7.1 Hz)], and four olefinic protons in a conjugated diene system [δ 7.26 (1H, dd, J = 15.0 and 10.1 Hz); 6.55 (1H, d, J = 15.0 Hz); 6.21 (2H, m)] (Table 1). The ^{13}C NMR spectrum of **1** when analyzed with the help of HSQC data displayed signals for 18 carbon atoms and indicated the presence of a ketone carbonyl (δ 195.1), an ester carbonyl (δ 171.1), six aromatic carbons of which

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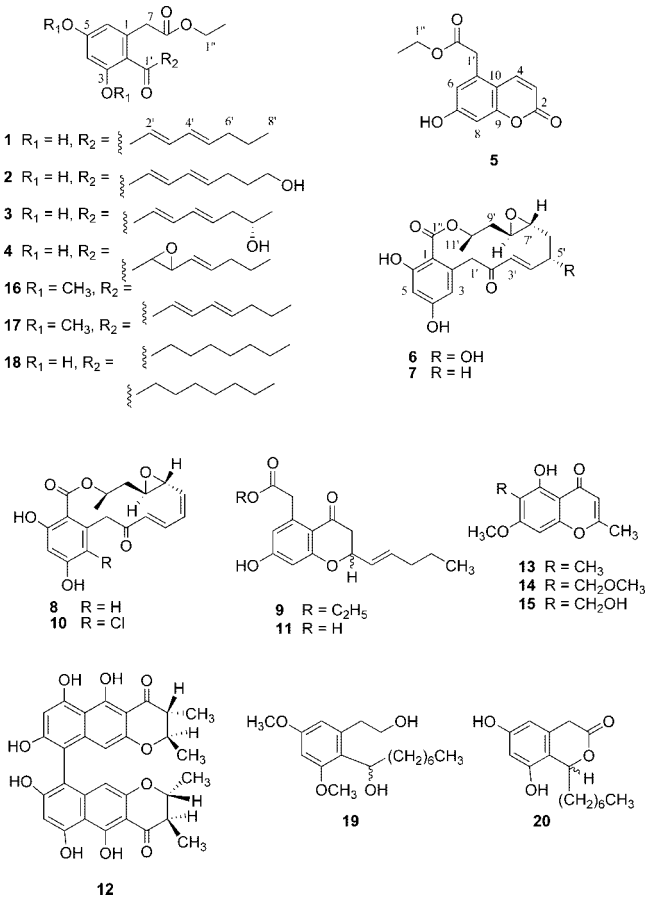


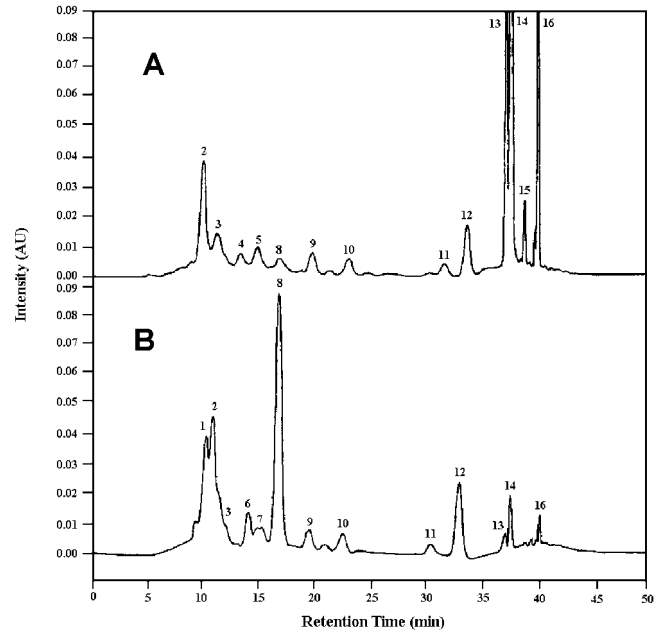


Figure 1. HPLC profiles of crude EtOAc extracts of *P. quadriseptata* cultured in (A) PDB medium made up in distilled water and (B) PDB medium made up in tap water. Peak identities (compounds corresponding to peaks 4, 9, 10, 11, 13, and 15 have not been isolated from *P. quadriseptata*, and therefore the identities of these peaks were not determined): peak 1 = 5′-hydroxymonocillin III (**6**); 2 = cytosporone G (**2**); 3 = cytosporone H (**3**); 5 = quadriseptin A (**5**); 6 = monocillin III (**7**); 7 = cytosporone I (**4**); 8 = monocillin I (**8**); 12 = aposphearin C (**11**); 14 = cytosporone F (**1**); 16 = aposphearin B (**10**).

two are oxygenated (δ 163.6 and 160.6), four protonated olefinic carbons, four methylene of which one is oxygenated (δ 61.4), and two methyl carbons (Table 2). The ^{1}H−^{1}H correlations observed for **1** in the DQF-COSY spectrum suggested the presence of the spin systems −OCH_2CH_3 (see above) and −CH═CH−CH═CH−CH_2−CH_2−CH_3. The cross-peaks between δ_H 6.55 (H-2′) and δ_C 129.0 (C-4′), δ_H 7.26 (H-3′) and δ_C 146.9 (C-5′), and δ_H 6.21 (H-4′) and δ_C 35.3 (C-6′) in the HMBC spectrum (Figure 2) established the connectivity of olefinic carbons, and the correlations between δ_H 3.77 (H-7) and δ_C 171.1 (C-8), δ_H 3.77 (H-7) and δ_C 111.6 (C-6), and δ_H 4.16 (H-1″) and δ_C 171.1 (C-8) suggested the presence of a −$CH_2CO_2CH_2CH_3$ moiety at C-1. The HMBC correlations between δ_H 6.55 (H-2′) and δ_C 116.9 (C-2) suggested the attachment of the ketone carbonyl to C-2 of the aromatic ring. The presence of two phenolic OH groups and two double bonds in **1** was confirmed by methylation with CH_3I/K_2CO_3 to afford the dimethyl derivative **16** and catalytic hydrogenation of **16** to yield its tetrahydro derivative, 3,5-di-*O*-methylcytosporone B (**17**). Treatment of **17** with $NaBH_4$ afforded 3,5-dimethoxy-2-(1′-hydroxyoctyl)phenethyl alcohol (**19**). Thus, the structure of **1** was suspected to be related to cytosporone B (**18**), and this was confirmed by the catalytic hydrogenation of **1** to yield **18** with spectroscopic data identical with those reported for cytosporone B.[10] Finally, the treatment of **1** with $NaBH_4/CH_3OH/NaOH$ afforded a product that was identified as cytosporone C (**20**).[10] On the basis of the foregoing evidence, the structure of cytosporone F was elucidated as 3,5-dihydroxy-2-(1′-oxoocta-2′*E*,4′*E*-dienyl)benzeneacetic acid ethyl ester (**1**).

The molecular formula of cytosporone G (**2**) was determined as $C_{18}H_{22}O_6$ from its HRFABMS and ^{13}C NMR data. IR absorption bands at 3429, 1720, and 1654 cm^{-1} suggested the presence of OH, and ester and ketone carbonyl groups. ^{1}H and ^{13}C NMR spectra of **2** (Tables 1 and 2, respectively) resembled those of **1** except that the signals due to the terminal CH_3 group [δ_H 0.91 t (J = 7.4 Hz); δ_C 13.7] are replaced with a CH_2 group and the chemical shift data [δ_H 3.56 t (J = 6.4 Hz); δ_C 61.6] suggested that in **2** this carbon is oxygenated. Cytosporone G was therefore identified as 3,5-dihydroxy-2-(8′-hydroxy-1′-oxoocta-2′*E*,4′*E*-dienyl)benzeneacetic acid ethyl ester (**2**). HRFABMS, ^{13}C NMR, and DEPT data suggested the molecular formula $C_{13}H_{12}O_5$ for quadriseptin A (**5**). It exhibited a UV spectrum (λ_{max} 322 and 215 nm) characteristic of a 7-oxygenated coumarin.[12] The IR spectrum of **5**, with absorption bands at 3571, 1770, and 1705 cm^{-1}, indicated the presence of OH, α,β-unsaturated lactone (of a coumarin), and ester carbonyl groups, respectively. The ^{1}H NMR spectrum of **5** indicated the presence of a −$CH_2CO_2CH_2CH_3$ moiety [δ_H 3.91 (2H, s), 4.11 (2H, q, J = 7.1 Hz), and 1.19 (3H, t, J = 7.1 Hz)] in addition to an OH (δ 9.43), two *meta*-coupled aromatic protons at δ 6.80 and 6.67 (each d, J = 2.2 Hz), and two olefinic protons at δ 8.00 (d, J = 9.7 Hz) and 6.17 (d, J = 9.7 Hz). The ^{13}C and DEPT NMR spectra of **5** confirmed the presence of an α,β-unsaturated lactone carbonyl (δ 161.3), an ester carbonyl (δ 170.8), six aromatic carbons of which two are oxygenated (δ 160.7 and 157.5) and two are protonated (δ 115.9 and 102.6 d), two protonated olefinic carbons (δ 141.8 and 112.5), one methylene (δ 38.2) sandwiched by an aromatic carbon and a carbonyl, one oxygenated methylene (δ 61.5), and one methyl carbon (δ 14.4). The foregoing data suggested the structure of quadriseptin A as 5-(ethoxycarbonylmethyl)-7-hydroxy-2*H*-1-benzopyran-2-one (**5**). The remaining compounds present in this extract were identified as monocillin I (**8**)[8] and aposphaerin B (**9**)[13] by comparison of their spectral data with those reported in the literature. The desethyl derivative of **9** [aposphaerin C (**11**)] and monocillin I (**8**) have previously been encountered in *P. quadriseptata* cultured in PDA and PDB made up in tap water.[8,9] The occurrence of aposphaerin B and the related octaketide, cavoxinone, as racemic mixtures has led to the proposition that they have been formed by nonenzymatic cyclizations of the corresponding olefinic open-chain precursors.[13] The co-occurrence of aposphaerin B (**9**) and cytosporone F (**1**) in the same extract

Table 1. ^{1}H NMR Data (500 MHz) for Compounds **1**–**4**

	δ_H multiplicity (*J* in Hz)			
position	**1**[a]	**2**[b]	**3**[b]	**4**[b]
4	6.31 s	6.34 d (2.3)	6.34 d (2.2)	6.35 d (2.1)
6	6.31 s	6.36 d (2.3)	6.36 d (2.2)	6.38 d (2.1)
7	3.77 s	3.75 s	3.70 s	3.93 s
2′	6.55 d (15.0)	6.61 d (15.1)	6.61 d (15.1)	4.87 d (6.2)
3′	7.26 dd (15.0, 10.1)	7.11 dd (15.1, 10.3)	7.11 dd (15.1, 10.6)	3.54 t (6.2)
4′	6.21 m	6.30 dd (15.1, 10.3)	6.29 dd (15.1, 10.6)	5.92 m
5′	6.21 m	6.27 dt (15.1, 6.2)	6.28 dt (15.1, 6.9)	5.72 dd (15.3, 6.2)
6′	2.16 m	2.29 m	2.33 m	2.67 dd (16.4, 11.7) 2.52 dd (16.4, 3.3)
7′	1.45 dq (14.7,7.4)	1.64 p (6.4)	3.84 m	1.61 m
8′	0.91 t (7.4)	3.56 t (6.4)	1.14 d (6.1)	1.28 t (7.1)
1″	4.16 q (7.1)	4.05 q (7.1)	4.05 q (7.1)	4.04 q (7.1)
2″	1.25 t (7.1)	1.18 t (7.1)	1.18 t (7.1)	1.19 t (7.1)
3-OH	11.51 (s)	-[c]	-[c]	-[c]

[a] In $CDCl_3$. [b] In acetone-d_6. [c] Signal not observed in acetone-d_6.

Table 2. ^{13}C NMR Data (125 MHz) for Compounds **1**–**4**

	δ_C			
position	**1**[a,c]	**2**[b,c]	**3**[b,c]	**4**[b,c]
1	136.5 C	135.3 C	137.6 C	138.2 C
2	116.9 C	119.2 C	119.3 C	114.9 C
3	163.6 C	161.3 C	161.3 C	164.3 C
4	102.7 CH	102.5 CH	102.5 CH	103.3 CH
5	160.6 C	161.1 C	161.4 C	162.0 C
6	111.6 CH	111.8 CH	111.8 CH	113.2 CH
7	40.9 CH_2	40.4 CH_2	40.4 CH_2	41.4 CH_2
8	171.1 C	171.2 C	171.2 C	172.6 C
1′	195.1 C	195.4 C	195.4 C	191.7 C
2′	128.0 CH	130.5 CH	130.6 CH	62.2 CH
3′	144.7 CH	143.9 CH	142.8 CH	43.4 CH
4′	129.0 CH	137.3 CH	131.7 CH	127.8 CH
5′	146.9 CH	145.6 CH	143.8 CH	134.7 CH
6′	35.3 CH_2	32.8 CH_2	43.8 CH_2	31.5 CH_2
7′	21.9 CH_2	25.4 CH_2	67.2 CH	28.6 CH_2
8′	13.7 CH_3	61.6 CH_2	23.6 CH_3	14.1 CH_3
1″	61.4 CH_2	61.0 CH_2	61.1 CH_2	61.2 CH_2
2″	14.2 CH_3	14.5 CH_3	14.5 CH_3	14.2 CH_3

[a] In $CDCl_3$. [b] In acetone-d_6. [c] Multiplicity from DEPT.

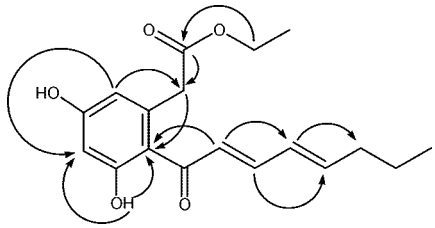
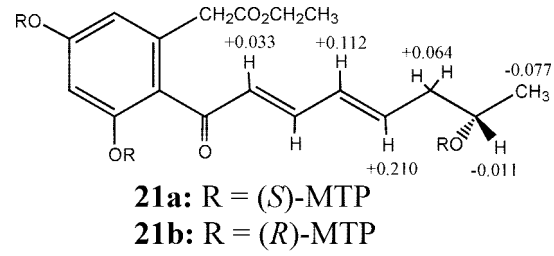


Figure 2. Selected HMBC correlations for **1**.

suggested that **9** may be an artifact originating from **1** by a Michael-type addition of its phenolic OH to the enone moiety during the isolation process. This was confirmed by the treatment of **1** with *p*-TSA, which resulted in the formation of **9**.

Fractionation of the EtOAc extract derived from *P. quadriseptata* cultured in PDB made up with tap water involving reversed-phase chromatography and silica gel preparative TLC afforded cytosporones F−I (**1**−**4**), compound **6**, monocillin III (**7**), and monocillin I (**8**). Comparison (HPLC, TLC, LR-MS, and ^{1}H NMR) with samples obtained above allowed the identification of cytosporones F (**1**) and G (**2**) and monocillin I (**8**). Monocillin III (**7**) was identified by comparison of its spectroscopic (LR-MS and ^{1}H NMR) data with those reported in the literature.[14] The molecular formula of cytosporone H (**3**) was established as $C_{18}H_{22}O_6$ from its HRFABMS and ^{13}C NMR data. Its IR absorption bands at 3283, 1712, and 1613 cm^{-1} suggested the presence of OH, ester, and ketone carbonyls, respectively. The molecular formula of **3** indicated that it is isomeric with cytosporone G (**2**). However, the ^{1}H and



21a: R = (*S*)-MTP
21b: R = (*R*)-MTPA

Figure 3. $\Delta\delta$ value [$\Delta\delta$ (in ppm) = $\delta_S - \delta_R$] obtained for (*S*)- and (*R*)-MTPA esters (**21a** and **21b**, respectively) of cytosporone H (**3**).

^{13}C NMR spectra of **3** (Tables 1 and 2, respectively) resembled those of cytosporone F (**1**) except that in **3** signals due to the 7′-CH_2 are replaced with an oxygenated CH [δ_H 3.84 m; δ_C 67.2], suggesting the presence of a $-CH_2CH(OH)CH_3$ moiety in **3**. Reaction of **3** with (*R*)- and (*S*)-α-methoxy-α-trifluoromethylphenylacetic (MTP) acid chlorides afforded (*S*)- and (*R*)-MTP esters **21a** and **21b**, respectively (Figure 3). Analysis of $\Delta\delta$ values confirmed the *R* absolute configuration for C-7′ (Figure 3). The structure of cytosporone H was thus established as 3,5-dihydroxy-2-(7′*R*-hydroxy-1′-oxoocta-2′*E*,4′*E*-dienyl)benzeneacetic acid ethyl ester (**3**). Cytosporone I (**4**) was determined to have the molecular formula $C_{18}H_{22}O_6$ by HRFABMS, which was consistent with its ^{13}C NMR data and indicated eight degrees of unsaturation. It had IR bands at 3417, 1737, and 1624 cm^{-1}, suggesting the presence of OH, ester, and ketone carbonyl groups. The ^{1}H and ^{13}C NMR data (Tables 1 and 2, respectively) showed very close resemblance to those of cytosporones F−H (**1**−**3**). The ^{13}C NMR spectrum of **4** analyzed with the help of the DEPT spectrum revealed the presence of two methyl, four methylene of which one is oxygenated (δ 61.2), six methine, and four quaternary carbons in addition to the two carbonyl carbons. Of the six methines, two each were aromatic (δ_C 103.3 and 113.2), olefinic (δ_C 127.8 and 134.7), and oxygenated aliphatic (δ_C 62.2 and 43.4) carbons. The ^{1}H NMR spectrum of **4** had two olefinic protons [δ_H 5.92 (m) and 5.72 (dd, J = 15.3 and 6.2 Hz)] and two protons attached to oxygenated carbons at δ_H 4.87 (d, J = 6.2 Hz) and 3.54 (t, J = 6.2 Hz). Comparison of the ^{1}H and ^{13}C NMR data of **4** with those of cytosporone F (**1**) suggested that in **4** the 2′(3′)-double bond is replaced with an oxirane ring. The structure of cytosporone G was thus established as 3,5-dihydroxy-2-(2′,3′-epoxy-1-oxoocta-4′*E*-enyl)benzeneacetic acid ethyl ester (**4**).

Compound **6**, isolated as a white, amorphous solid, was determined to have the molecular formula $C_{18}H_{20}O_7$ by a combination of HRFABMS and ^{13}C NMR data and indicated nine degrees of unsaturation. Its IR spectrum with absorption bands at 3400, 1718, and 1637 cm^{-1} suggested the presence of OH, lactone carbonyl, and α,β-unsaturated ketone carbonyl groups. Comparison

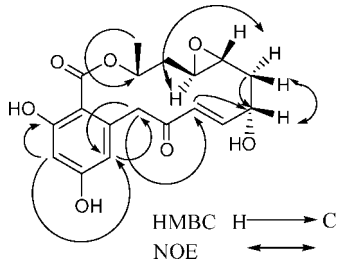


Figure 4. Selected HMBC and NOE correlations for **6**.

of the ^{1}H NMR and ^{13}C NMR data of **6** with those of monocillin III (**7**) obtained above indicated that they are structurally related, and this combined with the presence of an additional oxygen atom in **6** compared with **7** as evident from their molecular formulas suggested that **6** may be an oxygenated derivative of monocillin III. In the ^{13}C NMR spectrum of **6**, the chemical shift of C-5′ (δ 70.3) indicated that it is oxygenated, and this was confirmed by the presence of strong correlations between H-6′ (δ 2.53 and 1.28) and C-5′ (δ 70.3) and between H-3′ (δ 6.10) and C-5′ (δ 70.3) in its HMBC spectrum (Figure 4). The gross structure of **6** was therefore suspected to be 5′-hydroxymonocillin III. The relative stereochemistry of **6** was determined with the help of ^{1}H NMR coupling constants and pulse-field gradient 1D NOE experiments (Figure 4). The large coupling constant (16.2 Hz) observed for H-3′ and H-4′ suggested the *E* configuration for the 3′(4′)-double bond as in monocillin III (**7**).[14] The disposition of the oxirane ring was determined as *trans* from the pulse-field gradient NOE experiment, which showed an enhancement of the ^{1}H signal at δ 2.62 (H-8′) on irradiation of the signal at δ 1.28 (H-6′α), suggesting that these protons are on the same side of the ring. Enhancement of the ^{1}H signal at δ 4.44 (H-5′) on irradiation of the signal at δ 2.53 (H-6′β) indicated that H-5′ and H-6′β have the same orientation, confirming the structure of **6** as 5′-hydroxymonocillin III. Attempted preparation of Mosher's esters of **6** to determine its absolute stereochemistry failed probably due to its ready dehydration under the basic conditions used for this reaction.

Intrigued by the dependence of *P. quadriseptata* on the quality of the water for the production of its major metabolites, we analyzed the tap water used in the culture medium for metal ions, and it was found to contain a significant amount of Cu^{2+} (0.15 ppm) compared to other heavy metals (Cd^{2+} and Cr^{3+}).[16] Although the direct effect of heavy metal ions in the production of secondary metabolites in microorganisms is hitherto unknown, the influence of Cu^{2+} on superoxide dismutase (SOD) activity of *Aspergillus niger* strain B-77 has recently been reported.[17] It has been suggested that cellular physiology correlating with the enhancement of SOD activity in the presence of Cu^{2+} is indicative of the role this enzyme plays in the cellular adaptation to environmental (oxidative and temperature) stress.[17] We have recently reported the effect of monocillin I (**8**), the major metabolite produced by *P. quadriseptata* when cultured in tap water, on the stress-related protein Hsp90[8b] and its ability to confer thermotolerance to *Arabidopsis thaliana*.[18] It was of interest, therefore, to investigate the effect of Cu^{2+}, Cd^{2+}, and Cr^{3+} ions on the production of **8** in liquid media. *P. quadriseptata* was thus cultured in PDB made up in distilled water containing nontoxic concentrations of these ions. The EtOAc extracts of the supernatants of these cultures when analyzed by HPLC for the content of monocillin I (**8**) clearly indicated that these metal ions had a significant influence on its production (Figure 5).

C. chiversii, an endophytic fungal strain known to produce the Hsp90 inhibitor radicicol (**10**) as the major metabolite when cultured in PDA,[8b] was also investigated for the effect of culture conditions on the production of **10** and other metabolites. The organism was cultured for 7 days separately in PDB made up with distilled water and tap water and filtered, and the resulting culture supernatants and mycelia were separately extracted with EtOAc. TLC and HPLC

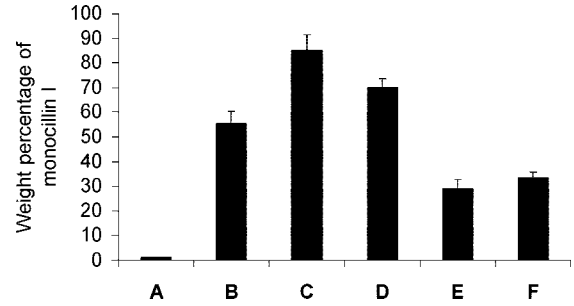


Figure 5. Effect of heavy metal ions on the production of monocillin I (**8**) by *P. quadriseptata* cultured in PDB medium made up in (A) distilled water, (B) tap water, (C) 0.50 mM $CuSO_4$ in distilled water, (D) 0.50 mM $ZnSO_4$ in distilled water, (E) 0.125 mM $Cd(NO_3)_2$ in distilled water, and (F) 0.0125 mM $K_2Cr_2O_7$ in distilled water. Presented are the mean value and standard deviation (SD) of triplicate determinations.

Table 3. Antiproliferative Activities of 5′-Hydroxymonocillin III (**6**) and Monocillin III (**7**) against a Panel of Three Tumor Cell Lines[a]

	cell line[b]		
compound	NCI-H460	MCF-7	SF-268
6	0.30	0.15	0.55
7	0.50	0.40	0.30
monocillin I (**8**)	0.35	0.60	0.72
doxorubicin	0.01	0.07	0.04

[a] Results are expressed as IC_{50} values in μM. [b] Key: NCI-H460 = human non small cell lung cancer; MCF-7 = human breast cancer; SF-268 = human CNS cancer (glioma).

analysis of these extracts indicated that this organism failed to produce radicicol (**10**) in liquid culture (data not shown). Most of the major metabolites were present in the mycelial extract of *C. chiversii* grown in both tap water and distilled water, and these were isolated by solvent−solvent partition followed by column chromatography to afford chaetochromin A (**12**) as the major constituent together with eugenetin (**13**) and 6-methoxymethyleugenin (**14**). Chaetochromin A was identified by comparison of its ^{1}H NMR, ^{13}C NMR, and MS data with those reported,[19–21] whereas **12** and **13** were identified by comparison with those obtained previously from the same organism.[9] Chaetochromin A (**12**) has previously been reported from *Chaetomium gracile*[19] and *C. virescens*.[20] This constitutes the first report of its occurrence in *C. chiversii*.

We have previously reported the antiproliferative and Hsp90 inhibitory activities of monocillin I (**8**) and radicicol (**10**).[8b] Compounds **1**−**7** and chaetochromin A (**12**) encountered in this study were evaluated for their ability to induce heat shock response and inhibit proliferation of three cancer cell lines [NCI-H460 (non small cell lung), MCF-7 (breast), and SF-268 (CNS glioma)]. Monocillin I and/or radicicol were used in these assays as positive controls to assess the relative activities of the compounds tested. In the former assay, cells were exposed to serial dilutions of test compounds for 72 h in RPMI 1640 media supplemented with 10% fetal bovine serum, and when cell viability was evaluated by the MTT assay,[22] only 5′-hydroxymonocillin III (**6**) and monocillin III (**7**) were found to inhibit proliferation/survival of the cell lines used; other compounds did not show any detectable inhibition at concentrations up to 5.0 μM. The concentrations resulting in 50% inhibition of cell proliferation/survival as measured by the MTT assay (IC_{50}) were found to range between 0.15 and 0.55 μM. It is significant that compared with monocillin I (**8**), 5′-hydroxymonocillin III (**6**) showed selective activity toward the breast cancer (MCF-7) cell line (Table 3). When compounds **1**−**7** and chaetochromin A (**12**) were tested in the heat shock induction assay using the reporter 3T3-Y9/B12 cells,[8b] only 5′-hydroxymonocillin III (**6**)

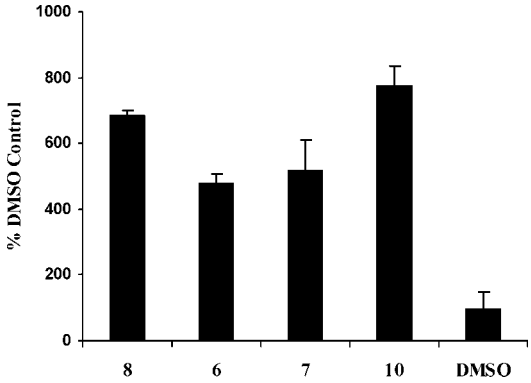


Figure 6. Cell-based heat shock induction assay (HSIA) of monocillin I (**8**), 5′-hydroxymonocillin III (**6**), and monocillin III (**7**). Radicicol (**10**) and DMSO were used as positive and negative control, respectively. All samples were tested at 1.0 μM concentration. The mean value and standard deviation (SD) of triplicate determinations are presented, expressed as a percentage of the negative control.

and monocillin III (**7**) showed activity at a concentration less than 5.0 μM. The ability of the monocillin analogues **6** and **7** to induce heat shock response was found to be slightly less than that observed for monocillin I (**8**) and radicicol (**10**) in this assay (Figure 6). Further studies are in progress to understand the effect of heavy metal ions in the production of metabolites by these and other plant-associated fungal strains.

Experimental Section

General Experimental Procedures. Optical rotations were measured with a Jasco DIP-370 digital polarimeter using MeOH as solvent. IR spectra were recorded on a Shimadzu FTIR-8300 spectrometer in KBr disks, and UV spectra in MeOH on a Shimadzu UV-1601 spectrometer. Analytical HPLC was performed on a Hitachi instrument equipped with an L-6200A intelligent pump, an L-4500 diode array detector, and a P-6000 interface utilizing Hitachi model D-7000 chromatography data station software using a Kromasil 5 μm C-18 column (4.6 mm × 250 mm); injections were made with an AS-4000 intelligent autosampler, and the mobile phase consisted of a gradient of MeOH (60–100%) in water at a flow rate of 0.4 mL min^{-1}. 1D and 2D NMR spectra were recorded in $CDCl_3$, acetone-d_6, and pyridine-d_5 using residual solvents as internal standards on a Bruker DRX-500 instrument at 500 MHz for ^{1}H NMR and 125 MHz for ^{13}C NMR. The chemical shift values (δ) are given in parts per million (ppm), and the coupling constants are in Hz. LR-MS and HR-MS were recorded on Shimadzu LCMS QP8000α and JEOL HX110A spectrometers, respectively.

Cultivation, Extraction, and Isolation of Metabolites of *P. quadriseptata*. The rhizosphere fungus *P. quadriseptata* isolated from the Christmas cactus, *Opuntia leptocaulis* DC. (Cactaceae), as described previously,[8a] was grown on PDA for 14 days and used for inoculation of the liquid cultures. Mycelia were scraped out and mixed with sterile PDB (150 mL) and filtered through a 100 μm filter to separate spores from mycelia. The optical density of the filtrate containing fungal spores was adjusted to a value between 0.5 and 0.3 with sterile PDB, and this spore suspension (50 mL) was used to inoculate two Erlenmeyer flasks (2.0 L), each containing 1.0 L of PDB prepared with distilled water and PDB prepared with tap water. Flasks were shaken on a rotary shaker at 28 °C and 160 rpm. On day 14, mycelia of each culture were separated from the supernatant by filtration using Whatman No. 1 filter paper. The mycelia were dried by vacuum filtration, frozen at −80 °C, and lyophilized for 24 h. The supernatants from each experiment were neutralized to pH 7 and extracted separately with EtOAc (8 × 500 mL), and the EtOAc extracts were evaporated under reduced pressure to afford dark brown semisolids (208 and 388 mg, respectively, from culture media prepared with distilled water and tap water). A portion (120 mg) of the EtOAC extract obtained from the liquid culture prepared with distilled water was subjected to reversed-phase column chromatography on a column of LRP-2 (4.0 g) using a gradient of MeOH in water. Fifty-two fractions were collected and combined on the basis of their TLC profiles to yield 11 fractions [A (4.8 mg), B (7.4 mg), C (4.6 mg), D (3.4 mg), E (29.3 mg), F (12.8 mg), G (2.6 mg), H (1.6 mg), I (14.0 mg), J (1.8 mg), and K (12.0 mg)]. Of these, fraction E (29.0 mg) was purified by silica gel preparative TLC (CH_2Cl_2/MeOH, 95:5) to afford **1** (25.4 mg). Fractions A and F were purified separately by reversed-phase preparative TLC on RP-18 (MeOH/H_2O, 75:25) to obtain **5** (3.0 mg) and **2** (9.8 mg), respectively. The combined fractions B, C, and D (15.4 mg) on further purification by preparative silica gel TLC (CH_2Cl_2/MeOH, 97:3) yielded **8** (3.0 mg) and **9** (11.3 mg). A portion (380 mg) of the EtOAc extract obtained from the liquid culture prepared with tap water was subjected to reversed-phase column chromatography on a column of LRP-2 (12.0 g) using a gradient of MeOH in water. Seventy-five fractions were collected and combined on the basis of their TLC profiles to yield four fractions [L (81.0 mg), M (76.2 mg), N (141.0 mg), and O (55.2 mg)]. Of these, fraction M was purified by preparative silica gel TLC (CH_2Cl_2/MeOH, 90:10) followed by reversed-phase (RP-18) preparative TLC (MeOH/H_2O, 60:40) to furnish **2** (2.9 mg), **3** (3.0 mg), **4** (0.9 mg), **6** (3.5 mg), and **7** (1.5 mg). A portion (20.0 mg) of fraction N was purified by preparative silica gel TLC (Et_2O/MeOH, 99:1) to obtain **7** (1.4 mg) and **8** (6.7 mg). A portion (10.0 mg) of the combined fraction O when purified by preparative silica gel TLC (CH_2Cl_2/MeOH, 95:5) afforded a further quantity of **8** (1.0 mg).

Cytosporone F (1): pale brown oil; UV (EtOH) λ_{max} (log ε) 288 (7.47), 208 (8.32) nm; IR (KBr) ν_{max} 3369, 2960, 1732, 1724 1614, 1593, 1461, 1319, 1267, 1161, 1020, 1002 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 319.1552 [M + 1]$^+$ (calcd for $C_{18}H_{23}O_5$, 319.1546).

Cytosporone G (2): pale brown oil; UV (EtOH) λ_{max} (log ε) 287 (7.58), 206 (7.44) nm; IR ν_{max} 3429, 1720, 1654, 1029, 856 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 335.1502 [M + 1]$^+$ (calcd for $C_{18}H_{23}O_6$, 335.1495).

Cytosporone H (3): yellow oil; $[\alpha]_D^{25}$ −13.04 (*c* 0.13, MeOH); UV (EtOH) λ_{max} (log ε) 305 (5.55), 298 (5.09), 204 (5.23) nm, IR ν_{max} 3283, 2922, 1712, 1613 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 335.1497 [M + 1]$^+$ (calcd for $C_{18}H_{23}O_6$, 335.1495).

Cytosporone I (4): colorless, amorphous solid; UV (EtOH) λ_{max} (log ε) 309 (5.39), 277 (4.33), 217 (4.22), 201 (5.29) nm; IR ν_{max} 3417, 2922, 1737, 1624, 1577, 1542, 1463, 1425 cm^{-1}; ^{1}H and ^{13}C NMR data, see Tables 1 and 2, respectively; HRFABMS *m*/*z* 335.1509 [M + 1]$^+$ (calcd for $C_{18}H_{23}O_6$, 335.1495).

Quadriseptin A (5): colorless, amorphous solid; UV (EtOH) λ_{max} (log ε) 322 (6.60), 258 (6.50), 215 (6.42) nm; IR ν_{max} 3571, 1770, 1705, 1670, 829 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 9.43 (1H, s, OH), 8.00 (1H, d, J = 9.7 Hz, H-4), 6.80 (1H, d, J = 2.2 Hz, H-6), 6.67 (1H, d, J = 2.2 Hz, H-8), 6.17 (1H, d, J = 9.7, H-3), 4.11 (2H, q, J = 7.1 Hz, H-1″), 3.91 (2H, s, H-1′), 1.19 (3H, t, J = 7.1 Hz, H-2″); ^{13}C NMR (125 MHz, acetone-d_6) δ 170.8 (C, C-2′), 161.3 (C, C-2), 160.7 (C, C-7), 157.5 (C, C-9), 141.8 (CH, C-4), 135.7 (C, C-5), 115.9 (CH, C-6), 112.5 (CH, C-3), 111.9 (C, C-10), 102.6 (CH, C-8), 61.5 (CH_2, C-1″), 38.2 (CH_2, C-1′), 14.4 (CH_3, C-2″); HRFABMS *m*/*z* 249.0773 [M + 1]$^+$ (calcd for $C_{13}H_{13}O_5$, 249.0763).

5′-Hydroxymonocillin III (6): white, amorphous solid; $[\alpha]_D^{25}$ +40.98 (*c* 0.18, MeOH); UV (EtOH) λ_{max} (log ε) 304 (5.40), 264 (4.84), 216 (4.75), 198 (5.25) nm; IR ν_{max} 3400, 1718, 1637, 1579 cm^{-1}; ^{1}H NMR (500 MHz, acetone-d_6) δ 11.93 (1H, s, OH), 6.82 (1H, dd, J = 16.2 and 8.0 Hz, H-4′), 6.32 (2H, s, H-3 and H-5), 6.10 (1H, d, J = 16.2 Hz, H-3′), 5.25 (1H, m, H-10′), 4.72 (1H, d, J = 17.6 Hz, H-1′a), 4.44 (1H, m, H-5′), 3.75 (1H, d, J = 17.6 Hz, H-1′b), 2.89 (1H, m, H-7′), 2.62 (1H, dt, J = 10.0 and 2.9 Hz, H-8′), 2.53 (1H, dt, J = 12.5 and 3.9 Hz, H-6′β), 2.05 (1H, m, H-9′a), 1.72 (1H, dt, J = 15.9 and 4.9 Hz, H-9′b), 1.43 (3H, d, J = 6.4 Hz, H-11′), 1.28 (1H, m, H-6′α); ^{13}C NMR (125 MHz, acetone-d_6) δ 197.2 (C, C-2′), 172.0 (C, C-1″), 167.0 (C, C-4), 163.3 (C, C-6), 150.2 (CH, C-4′), 141.0 (C, C-2), 129.2 (CH, C-3′), 113.7 (CH, C-3), 105.8 (C, C-1), 102.8 (CH, C-5), 72.4 (CH, C-10′), 70.3 (CH, C-5′), 55.9 (CH, C-7′), 54.6 (CH, C-8′), 48.4 (CH_2, C-1′), 41.4 (CH_2, C-6′), 36.9 (CH_2, C-9′), 18.1 (CH_3, C-11′); HRFABMS *m*/*z* 349.1285 [M + 1]$^+$ (calcd for $C_{18}H_{21}O_7$ 349.1287).

Methylation of Cytosporone F. Methyl iodide (0.5 mL) and K_2CO_3 (10.0 mg) were added to a stirred solution of **1** (10.0 mg) in acetone (0.5 mL) at 0 °C. After 5 min at 0 °C, the ice bath was removed and the reaction mixture was stirred at room temperature until the starting material disappeared (TLC control). It was then filtered, the solvent

was removed under reduced pressure, and the crude product was purified by preparative TLC (silica gel) using CH_2Cl_2/MeOH (95:5) as eluant to give 3,5-di-*O*-methylcytosporone F (8.6 mg).

3,5-Di-*O*-methylcytosporone F (16): pale brown oil; UV (EtOH) λ_{max} (log ε) 279 (5.68), 213 (5.57) nm; IR ν_{max} 2925, 1735, 1602, 1460, 1157 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.94 (1H, dd, J = 15.4 and 10.8 Hz, H-3′), 6.41 (1H, d, J = 2.2 Hz, H-6), 6.38 (1H, d, J = 2.2 Hz, H-4), 6.35 (1H, d, J = 15.4 Hz, H-2′), 6.21 (1H, dd, J = 15.1 and 10.8 Hz, H-4′), 6.12 (1H, dt, J = 15.1 and 6.9 Hz, H-5′), 4.06 (2H, q, J = 7.1 Hz, H-1″), 3.80 (3H, s, OCH_3), 3.74 (3H, s, OCH_3), 3.55 (2H, s, H-7), 2.12 (2H, m, H-6′), 1.41 (2H, dq, J = 14.8 and 7.4 Hz, H-7′), 1.18 (3H, t, J = 7.1 Hz, H-2″), 0.89 (3H, t, J = 7.4 Hz, H-8′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 196.2 (C, C-1′), 171.1 (C, C-8), 161.2 (C, C-3), 158.6 (C, C-5), 145.4 (CH, C-5′), 143.4 (CH, C-3′), 134.6 (C, C-1), 130.3 (CH, C-2′), 129.3 (CH, C-4′), 113.9 (C, C-2), 107.3 (CH, C-6), 97.6 (CH, C-4), 60.8 (CH_2, C-1″), 55.8 (CH_3, OCH_3), 55.4 (CH_3, OCH_3), 38.7 (CH_2, C-7), 35.2 (CH_2, C-6′), 21.9 (CH_2, C-7′), 14.2 (CH_3, C-2″), 13.6 (C, C-8′); HRFABMS m/z 347.1860 $[M + 1]^+$ (calcd for $C_{20}H_{27}O_5$, 347.1858).

Catalytic Hydrogenation of 3,5-Di-*O*-methylcytosporone F (16). A solution of **16** (8.0 mg) in EtOH (0.5 mL) containing 10% Pd on carbon (1 mg) was stirred in an atmosphere of H_2 for 3 h (TLC control). The solution was filtered through a plug of cotton, and the solvent was evaporated under reduced pressure. The crude product was purified by preparative TLC (silica gel) using CH_2Cl_2/*i*-PrOH (99:1) as eluant to give 3,5-di-*O*-methylcytosporone B (**17**) (5.3 mg).

3,5-Di-*O*-methylcytosporone B (17): colorless oil; UV (EtOH) λ_{max} (log ε) 283 (6.00), 226 (5.97) nm; IR ν_{max} 2927, 1735, 1681, 1317, 1155 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.37 (1H, d, J = 2.3 Hz, H-6), 6.36 (1H, d, J = 2.3 Hz, H-4), 4.12 (2H, q, J = 7.1 Hz, H-1″), 3.79 (3H, s, OCH_3), 3.78 (3H, s, OCH_3), 3.59 (2H, s, H-7), 2.80 (2H, t, J = 7.4 Hz, H-2′), 1.62 (2H, m, H-3′), 1.26 (8H, m, H-4′−H-7′), 1.23 (3H, t, J = 7.1 Hz, H-2″), 0.86 (3H, t, J = 7.4 Hz, H-8′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 206.9 (C, C-1′), 171.2 (C, C-8), 161.2 (C, C-3), 158.7 (C, C-5), 134.4 (C, C-1), 124.3 (C, C-2), 107.7 (CH, C-6), 97.5 (CH, C-4), 60.9 (CH_2, C-1″), 55.6 (CH_3, OCH_3), 55.4 (CH_3, OCH_3), 44.5 (CH_2, C-2′), 38.9 (CH_2, C-7), 31.6 (CH_2, C-6′), 29.3 (CH_2, C-4′), 29.1 (CH_2, C-5′), 24.1 (CH_2, C-3′), 22.6 (CH_2, C-7′), 14.2 (CH_3, C-2″), 14.1 (CH_3, C-8′); HRFABMS m/z 351.2186 $[M + 1]^+$ (calcd for $C_{20}H_{31}O_5$, 351.2171).

Reduction of 3,5-Di-*O*-methylcytosporone F (17) with $NaBH_4$. $NaBH_4$ (5 mg) was added to a stirred solution of **17** (5.0 mg) in anhydrous MeOH (0.5 mL) at 0 °C. After 5 min, the ice bath was removed and the reaction mixture was stirred at room temperature until the starting material disappeared (TLC control). It was then filtered, the solvent was removed under reduced pressure, and the crude product was purified by preparative TLC (silica gel) using CH_2Cl_2/*i*-PrOH (99:1) as eluant to give 3,5-dimethoxy-2-(1′-hydroxyoctyl)phenethyl alcohol (**19**) (3.9 mg) as a colorless oil: UV (EtOH) λ_{max} (log ε) 283 (5.64), 227 (5.55), 214 (5.52) nm; IR ν_{max} 3427, 2925, 1602, 1460, 1147 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.37 (1H, d, J = 2.4, H-6), 6.32 (1H, d, J = 2.4 Hz, H-4), 4.83 (1H, dd, J = 8.6 and 5.5 Hz, H-1′), 3.83 (2H, t, J = 6.4 Hz, H-8), 3.82 (3H, s, OCH_3), 3.72 (3H, s, OCH_3), 2.89 (2H, t, J = 6.4 Hz, H-7), 1.95 (1H, m, H-2′a), 1.67 (1H, m, H-2′b), 1.26 (10H, m, H-3′−H-7′), 0.85 (3H, t, J = 7.4 Hz, H-8′); ^{13}C NMR (125 MHz, $CDCl_3$) δ 159.2 (C, C-3), 158.9 (C, C-5), 137.6 (C, C-1), 123.5 (C, C-2), 106.9 (CH, C-6), 97.7 (CH, C-4), 70.5 (CH_2, C-8), 63.5 (CH, C-1′), 55.4 (CH_3, OCH_3), 55.3 (CH_3, OCH_3), 37.8 (CH_2, C-7), 36.8 (CH_2, C-2′), 31.9 (CH_2, C-6′), 29.6 (CH_2, C-4′), 29.3 (CH_2, C-5′), 26.3 (CH_2, C-3′), 22.7 (CH_2, C-7′), 14.1 (CH_3, C-8′); HRFABMS m/z 293.2125 $[MH - H_2O]^+$ (calcd for $C_{18}H_{29}O_3$, 293.2117).

Conversion of Cytosporone F (1) to Cytosporone B (18). A solution of **1** (3.0 mg) in EtOH (0.5 mL) containing Pd on carbon (10%, 1 mg) was stirred in an atmosphere of H_2 for 2 h (TLC control). The solution was filtered through a plug of cotton, and the solvent was evaporated under reduced pressure to afford cytosporone B (**18**) as a white solid (3.0 mg). Its ^1H NMR, ^{13}C NMR, and MS data were consistent with those reported in the literature.[10]

Conversion of Cytosporone F (1) to Cytosporone C (20). A solution of **1** (3.0 mg) in 0.2 N NaOH (0.5 mL) was stirred with $NaBH_4$ (3.0 mg) at room temperature for 1 h (TLC control). The reaction mixture was acidified by adding an appropriate amount of 2 N HCl at 0 °C. After 5 min the ice bath was removed and the aqueous solution was extracted with EtOAc (2 × 10 mL). The solvent was evaporated under reduced pressure to afford cytosporone C (3.0 mg). Its ^1H NMR and MS data were consistent with those reported in the literature.[10]

Conversion of Cytosporone F (1) to Aposphaerin B (9). *p*-Toluenesulfonic acid (0.1 mg) was added to a stirred solution of **1** (2.0 mg) in dry toluene (0.5 mL), and the reaction mixture was heated at 65 °C for 2 h (TLC control). Toluene was removed under reduced pressure, and the crude mixture was dissolved in CH_2Cl_2/MeOH (90:10). The solution was passed through a short bed of silica gel using CH_2Cl_2/MeOH (9:1) (10 mL). The solvents were evaporated under reduced pressure to afford aphosphaerin B (2.0 mg). Its ^1H NMR and MS data were consistent with those reported in the literature.[13]

Monocillin III (7): white solid; ^1H NMR, ^{13}C NMR, and MS data were consistent with those reported in the literature.[14]

Preparation of the (*R*)- and (*S*)-MTPA Ester Derivatives of Cytosporone H (3) by a Convenient Mosher Ester Procedure.[15] Compound **3** (0.5 mg) was transferred into a clean NMR tube and was dried under vacuum. Pyridine-d_5 (0.6 mL) and (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (5 μL) were added into the NMR tube immediately under a stream of dry N_2, and then the NMR tube was shaken carefully to mix the sample and MTPA chloride evenly. The reaction mixture in the NMR tube was allowed to stand at room temperature, and the ^1H NMR was recorded after 6 h. ^1H NMR data of the (*S*)-MTPA ester derivative (**21a**) of **3** (500 MHz, pyridine-d_5): δ 6.459 (1H, d, J = 15.1 Hz, H-2′), 6.316 (1H, m, H-4′), 6.127 (1H, m, H-5′), 5.277 (1H, m, H-7′), 4.040 (2H, q, J = 7.1 Hz, H-1″), 3.905 (2H, s, H-7), 2.450 (2H, m, H-6′), 1.178 (3H, d, J = 6.2 Hz, H-8′), 1.051 (3H, t, J = 7.1 Hz, H-2″). In the manner described for **21a**, another portion of **3** (0.5 mg) was reacted in a second NMR tube with (*S*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (5 μL) at room temperature for 6 h using pyridine-d_5 (0.5 mL) as solvent to afford the (*R*)-MTPA ester derivative (**21b**) of **3**. ^1H NMR data of **21b** (500 MHz, pyridine-d_5): δ 6.426 (1H, d, J = 15.1 Hz, H-2′), 6.204 (1H, m, H-4′), 5.917 (1H, m, H-5′), 5.288 (1H, m, H-7′), 4.042 (2H, q, J = 7.1 Hz, H-1″), 3.905 (2H, s, H-7), 2.386 (2H, m, H-6′), 1.255 (3H, d, J = 6.2 Hz, H-8′), 1.053 (3H, t, J = 7.1 Hz, H-2″).

Effect of Metal Ions on the Production of Monocillin I (8). PDB (50 mL) was prepared with distilled water into each 250 mL conical flask, and appropriate weights of $CuSO_4$, $ZnSO_4$, $Cd(NO_3)_2$, and $K_2Cr_2O_7$, were added into each flask separately to obtain the final concentrations of 0.5 mM $CuSO_4$, 0.5 mM $ZnSO_4$, 0.125 mM $Cd(NO_3)_2$, and 0.0125 mM $K_2Cr_2O_7$. The spore solutions (1 mL) prepared as above were added into each flask and shaken on a rotary shaker at 160 rpm and 28 °C. The experiment with each metal ion concentration was carried out in triplicate. After two weeks the supernatant was extracted into EtOAc (3 × 50 mL) and concentrated to dryness. Each EtOAc extract was analyzed using reversed-phase HPLC, and the percentage of monocillin I produced by each culture was estimated.

Cultivation, Extraction, and Isolation of Metabolites of *Chaetomium chiversii*. A culture of *C. chiversii* grown on PDA for one week was used for inoculation. Mycelia were scraped out and mixed with sterile PDB (150 mL) and filtered through a 100 μm filter to separate spores from the mycelia. Absorbance of the spore solution was measured and adjusted to between 0.5 and 0.3. This spore solution (50 mL) was used to inoculate 2 L Erlenmeyer flasks containing 1.0 L of PDB prepared with filtered water. Flasks were shaken on a rotary shaker at 28 °C and 160 rpm for one week. On day 7, mycelia were separated from the supernatant by filtering through Whatman No. 1 filter paper. The supernatant was neutralized to pH 7 and extracted with EtOAc (8 × 500 mL). The lyophilized mycelia (20.0 g) were extracted with EtOAc (8 × 500 mL) and the EtOAc extracts evaporated under reduced pressure to afford a dark green semisolid (2.1 g). A portion (1.8 g) of this extract was partitioned between hexane and 80% aqueous MeOH. Evaporation of solvents under reduced pressure yielded hexane (1.02 g) and 80% aqueous MeOH (0.77 g) fractions. The 80% aqueous MeOH fraction was diluted with water to 50% aqueous MeOH and extracted with $CHCl_3$. Evaporation of $CHCl_3$ under reduced pressure yielded a brown semisolid (0.71 g), which was subjected to column chromatography on a column of LiChroprep DIOL (22 g, 25–40 μm) made up in hexane (100 mL) and eluted with hexane and mixtures of hexane/CH_2Cl_2 of increasing polarity, CH_2Cl_2, mixtures of CH_2Cl_2/MeOH of increasing polarity, and finally with MeOH. A total of 55 fractions (7.5 mL each) were collected, and fractions having similar TLC behavior were combined to give 17 subfractions. Of these, the subfraction 12 afforded chaetochromin A (**12**) (157.9 mg), the major

constituent of this extract. Subfractions 5 and 6 were purified by preparative silica gel TLC with CH_2Cl_2/*i*-PrOH (99:1) and CH_2Cl_2/MeOH (95:5) separately to give eugenetin (**13**) (20.2 mg) and 6-methoxymethyleugenin (**14**) (5.2 mg), respectively.

Chaetochromin A (12): yellow solid; MS and ^1H and ^{13}C NMR data were consistent with those reported in the literature.[21]

Cytotoxicity and Heat Shock Inhibition Assays. The tetrazolium-based cytotoxicity and heat shock induction assays were carried out as described previously.[8b]

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