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Five new isocoumarins from Sonoran desert plant-associated fungal strains *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii*☆

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Abstract—Five new isocoumarins, paraphaeosphaerins A–C and chaetochiversins A and B, biogenetically related to monocillin I and radicicol, have been isolated from solid agar cultures of *Paraphaeosphaeria quadriseptata* and *Chaetomium chiversii*, two fungal strains living in association with the Sonoran desert plants, *Opuntia leptocaulis* and *Ephedra fasciculata*, respectively. A new chroman-4-one, aposphaerin C, was also isolated from *P. quadriseptata*. Their structures and stereochemistry were elucidated using a combination of ^{1}H and ^{13}C homo- and hetero-nuclear 2D NMR techniques, ^{1}H NMR analysis of Mosher's esters, and chemical correlations.

1. Introduction

Recent studies have demonstrated that plant-associated fungi are rich sources of structurally diverse natural products, some with interesting biological activities.[2] In our continuing search for bioactive and/or novel metabolites of endophytic and rhizosphere fungi of the Sonoran desert plants, we have investigated EtOAc extracts of *Paraphaeosphaeria quadriseptata* occurring in the rhizosphere of the Christmas cactus (*Opuntia leptocaulis* DC.; Cactaceae) and *Chaetomium chiversii* endophytic in Mormon tea (*Ephedra fasciculata* A. Nels.; Ephedraceae). Here we report the isolation and characterization of five new isocoumarins, paraphaeosphaerins A–C (**1**–**3**) and chaetochiversins A and B (**4** and **5**) biogenetically related to monocillin I (**6**) and radicicol (**7**), a new chroman-4-one, aposphaerin C (**8**), and three known chromones, eugenetin (**9**), 6-methoxy-methyleugenin (**10**), and 6-hydroxy-methyleugenin (**11**). Previous studies of *P. quadriseptata* and *C. chiversii* have resulted in the isolation of cytotoxic and heat shock protein-90 (Hsp90) inhibitory β-resorcylic acid lactone macrolides, monocillin I (**6**) and radicicol (**7**), respectively.[1] Isolation of two 10-membered macrolides, modiolides A and B, from the marine-derived *Paraphaeosphaeria* sp. N-119 has recently been reported.[3]

2. Results and discussion

Liquid–liquid partitioning[4] of the EtOAc extract of a solid culture of *P. quadriseptata* followed by size exclusion chromatography of the $CHCl_3$ soluble fraction on Sephadex LH-20 and chromatographic separation of the fraction eluted with hexane/CH_2Cl_2 (1:4) and CH_2Cl_2/acetone (3:2) over a column of silica gel and preparative TLC furnished compounds **1**–**3** and **8**, in addition to the previously isolated monocillin I (**6**).[5]

Paraphaeosphaerin A (**1**) was obtained as a white amorphous solid that was analyzed for $C_{18}H_{18}O_6$ by a combination of HRFABMS and ^{13}C NMR spectroscopy and indicated ten degrees of unsaturation. Its UV spectrum with absorption maxima at 378, 360.5, 345.5, 330, and 270 nm was indicative of a conjugated chromophore and its IR spectrum with absorption bands at 3380, 1664, 1620, and 1570 cm^{-1} suggested the presence of OH/NH, α,β-unsaturated lactone carbonyl and olefinic groups. In the ^{1}H NMR spectrum of **1** (Table 1), in addition to other signals, a chelated OH (δ 11.12), a set of *meta*-coupled one-proton doublets [δ 6.24 and 6.32 (J=2.0 Hz)], and five olefinic/aromatic protons [δ 7.28 (dd, J=15.2, 11.4 Hz), 6.22 (s), 6.15 (dd, J=11.4, 10.2 Hz), 6.05 (d, J=15.2 Hz), and 5.56 (dd, J=10.2, 8.3 Hz)] were observed. The ^{13}C NMR spectrum of **1** (Table 2) indicated the presence of an α,β-unsaturated lactone/ester carbonyl, three oxygenated and nine non-oxygenated olefinic/aromatic carbons. In the HMBC spectrum, the proton at δ 6.22 (H-4) showed a correlation with a quaternary carbon at δ 99.9 (C-8a) and an aromatic carbon at δ 104.4 (C-5) bearing one of the *meta*-coupled protons [δ 6.32 (H-5)].

☆ See Ref. 1.

Keywords: *Paraphaeosphaeria quadriseptata*; *Chaetomium chiversii*; Endophytic and rhizosphere fungi; Paraphaeosphaerins; Chaetochiversins; Aposphaerin C; Structure elucidation.

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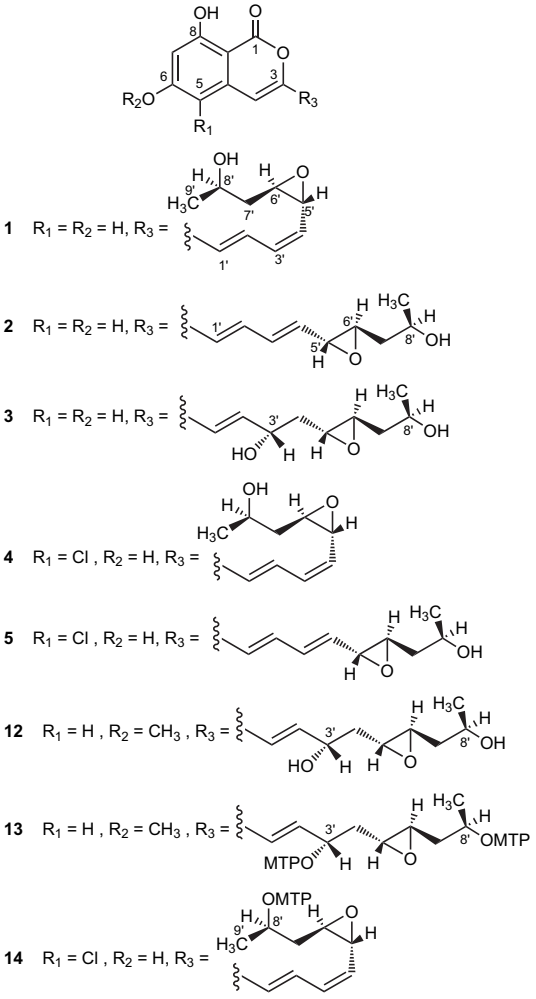


The proton at δ 6.32 (H-5) showed HMBC correlations with a quaternary carbon at δ 99.9 (C-8a), an aromatic carbon at δ 102.8 (C-7) bearing the remaining *meta*-coupled proton [δ 6.24 (H-7)], and an aromatic carbon at δ 107.6 (C-4) to which the proton at δ 6.22 is attached. The proton at δ 6.24 (H-7) showed HMBC correlations with the quaternary carbon at δ 99.9 (C-8a) and the aromatic carbon at δ 104.4 (C-5) (Fig. 1). These data suggested paraphaeosphaerin A to be a 6,8-dihydroxy-isocoumarin with a substituent at C-3 extending the conjugation of the C-3(4) double bond. The MS of **1** had a prominent ion at *m/z* 177 due to the dihydroxy-isocoumarin fragment further confirming this partial structure. In addition to the above low-field signals, the ^{1}H NMR spectrum of **1** indicated the presence of a tertiary methyl group at δ 1.25 (d, J=6.1 Hz), three methine protons at δ 4.69 (dd, J=8.3, 3.3 Hz), 4.42 (m), and 4.07 (dd, J=6.4, 3.3 Hz) attached to oxygenated carbons, and two methylene protons at δ 1.98 (ddd, J=13.0, 5.6, 2.4 Hz) and 1.68 (ddd, J=13.0, 9.4, 6.4 Hz). The ^{13}C NMR spectrum of **1** (Table 2) when analyzed with the help of edited HSQC spectra[6] showed the presence of one methyl, one methylene, and seven methine carbons in addition to the carbons encountered for the isocoumarin moiety. Of the seven methines, three were in the oxygenated region and four were in the olefinic region. These ^{1}H and ^{13}C NMR signals were assigned to the spin system –CH=CH–CH=CH–CH(O)–CH(O)–CH_2–CH(O)CH_3 (partial formula $C_9H_{12}O_3$) with the help of COSY, HSQC, and HMBC spectra. Since the dihydroxy-isocoumarin

Table 2. ^{13}C NMR data of compounds **1**–**5**

Position	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]
1	166.0 s	165.2 s	165.4 s	165.6 s	165.7 s
3	152.9 s	151.7 s	151.1 s	153.9 s	153.9 s
4	107.6 d	106.5 d	106.4 d	103.8 d	103.6 d
4a	137.4 s	139.4 s	139.3 s	138.1 s	137.0 s
5	104.4 d	103.5 d	102.3 d	107.1 s	107.9 s
6	166.3 s	165.6 s	165.7 s	161.7 s	162.9 s
7	102.8 d	102.2 d	103.6 d	103.2 d	103.3 d
8	164.5 s	164.5 s	163.2 s	162.7 s	163.2 s
8a	99.9 s	99.1 s	99.1 s	100.4 s	100.1 s
1′	125.2 d	122.8 d	120.8 d	125.1 d	123.3 d
2′	129.1 d	129.8 d	136.5 d	130.0 d	130.7 d
3′	129.6 d	132.4 d	68.9 d	129.4 d	134.3 d
4′	136.5 d	136.7 d	38.9 t	137.0 d	137.9 d
5′	84.1 d	86.7 d	55.3 d	84.0 d	83.6 d
6′	78.5 d	77.2 d	56.3 d	78.4 d	72.5 d
7′	43.8 t	41.9 t	40.3 t	43.7 t	44.3 t
8′	75.0 d	74.7 d	65.6 d	75.0 d	74.4 d
9′	21.5 q	20.8 q	22.8 q	21.4 q	21.8 q

[a] At 125 MHz ($CDCl_3$+CD_3OD).
[b] At 125 MHz ([$^2H_6$]-acetone), assignments were based on HSQC and HMBC experiments.

Table 1. ^{1}H NMR data of compounds **1**–**5**

Position	**1**[a]	**2**[a]	**3**[a]	**4**[b]	**5**[b]
4	6.22 s	6.20 s	6.21 s	6.84 s	6.82 s
5	6.32 d (2.0)	6.31 d (2.2)	6.30 d (2.2)		
7	6.24 d (2.0)	6.25 d (2.2)	6.23 d (2.2)	6.57 s	6.57 s
1′	6.05 d (15.2)	6.06 d (15.2)	6.22 d (15.5)	6.47 d (15.1)	6.43 d (15.3)
2′	7.28 dd (15.2, 11.4)	6.99 dd (15.2, 11.1)	6.52 dd (15.5, 5.0)	7.47 dd (15.1, 11.7)	7.01 dd (15.3, 10.9)
3′	6.15 dd (11.4, 10.2)	6.43 dd (15.2, 11.1)	4.45 m	6.28 dd (11.7, 10.6)	6.47 dd (15.3, 10.9)
4′	5.56 dd (10.2, 8.3)	5.99 dd (15.2, 6.3)	1.87 dt (14.1, 6.1)	5.69 dd (10.6, 8.5)	6.18 dd (15.3, 6.3)
4′			1.70 dt (14.1, 5.9)		
5′	4.69 dd (8.3, 3.3)	4.48 dd (6.3, 2.5)	2.80 dt (5.9, 2.2)	4.66 dd (8.5, 3.4)	4.49 dd (6.3, 3.1)
6′	4.07 dd (6.4, 3.3)	4.28 br t (6.3)	2.87 dt (5.9, 2.2)	4.12 dt (6.9, 3.4)	4.39 m
7′a	1.98 ddd (13.0, 5.6, 2.4)	2.11 ddd (13.2, 5.7, 1.1)	1.69 dt (14.0, 6.9)	1.99 ddd (12.8, 5.7, 2.7)	2.07 m
7′b	1.68 ddd (13.0, 9.4, 6.4)	1.67 ddd (13.2, 9.6, 6.3)	1.52 dt (14.0, 4.7)	1.71 ddd (12.8, 9.1, 6.9)	1.69 ddd (12.8, 9.5, 4.6)
8′	4.42 m	4.40 m	3.93 m	4.24 m	4.37 m
9′	1.25 d (6.1)	1.22 d (6.1)	1.60 d (5.3)	1.23 d (6.0)	1.19 d (6.1)
6-OH					
8-OH	11.12 s	11.20 s	10.95 s	11.13 s	11.19 s

[a] At 600 MHz ($CDCl_3$+CD_3OD), J values in hertz (in parenthesis).
[b] At 600 MHz (acetone-d_6), J values in hertz (in parenthesis).

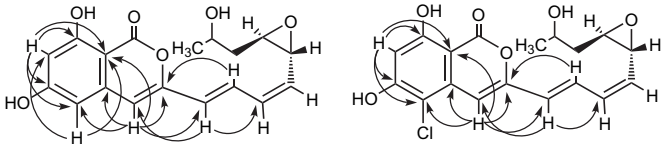
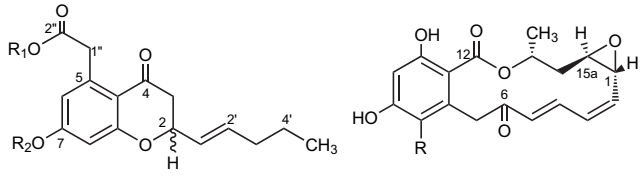


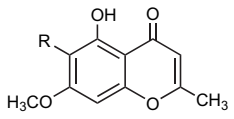
Figure 1. Selected HMBC correlations for paraphaeosphaerin A (**1**) and chaetochiversin A (**4**).

moiety accounts for $C_9H_5O_4$ with the molecular formula $C_{18}H_{18}O_6$ of **1**, the partial formula for the side-chain at C-3 should be $C_9H_{13}O_2$. In order to fulfill this requirement and the unsaturation number of 10 for paraphaeosphaerin A, an oxirane ring at C-5′(6′) and an OH substituent at C-8′ have been proposed. The cross peaks between δ_C 107.6 (C-4) and δ_H 6.32 (H-5) and 6.05 (H-1′), δ_C 125.2 (C-1′) and δ_H 6.22 (H-4) and 6.15 (H-3′) in the HMBC spectrum established the connectivity between the isocoumarin moiety and the side-chain. The relative configurations of the vicinal protons H-1′–H-6′ of the side-chain of paraphaeosphaerin A follow their characteristic coupling constants suggesting a trans relationship of the olefinic protons, H-1′ and H-2′ (J=15.2 Hz), and a cis relationship for H-3′ and H-4′ (J=10.2 Hz). The coupling constant of 3.3 Hz indicated a trans relationship of the protons (H-5′ and H-6′) on the oxirane ring.[7] The foregoing evidence suggested an isochromen-1-one structure **1** for paraphaeosphaerin A and this was confirmed by chemical transformation of monocillin I (**6**) to paraphaeosphaerin A (**1**). Treatment of **6** with potassium *tert*-butoxide in *tert*-butanol/DMF[8] yielded a product identical (TLC, MS, ^{1}H NMR, and ^{13}C NMR) with **1**. In addition to confirming the gross structure, formation of **1** from **6** also indicated that the absolute configurations of the three asymmetric centers of **1** to be identical with those of monocillin I (**6**). Since all three asymmetric centers of **6** have been determined by X-ray crystallographic analysis to have the *R* configuration,[9] the asymmetric carbons (C-5′, C-6′, and C-8′) of **1** must have the same *R* configuration. On the basis of the above data, the structure of paraphaeosphaerin A was elucidated as 3-(8′*R*-hydroxy-5′*R*,6′*R*-oxireno-1′*E*,3′*Z*-diennonyl)-6,8-dihydroxy-isochromen-1-one [6,8-dihydroxy-3-(8′*R*-hydroxy-5′*R*,6′*R*-oxirenonona-1′*E*,3′*Z*-dienyl)-1*H*-2-benzopyran-1-one] (**1**).



8 $R_1 = R_2 = H$
15 $R_1 = CH_2CH_3$, $R_2 = H$
16 $R_1 = R_2 = CH_3$

6 R = H
7 R = Cl

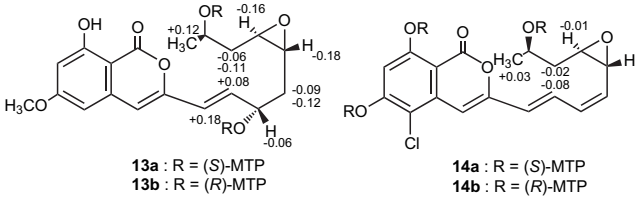


9 $R = CH_3$
10 $R = CH_2OCH_3$
11 $R = CH_2OH$

Paraphaeosphaerin B was obtained as an amorphous white solid. Its molecular formula was established as $C_{18}H_{18}O_6$ from HRFABMS and indicated ten degrees of unsaturation. The ^{1}H NMR spectral data of **2** (Table 1) were similar to those of **1**, except for the H-3′/H-4′ coupling constant (Table 1). The $J_{3',4'}$ of **2** was found to be 15.2 Hz (compared with 10.2 Hz observed for **1**) suggesting the *E* stereochemistry for the C-3′(4′) double bond in **2**. The stereochemical relationship between **1** and **2** was further confirmed by the treatment of **1** with iodine converting it into its more stable *E* isomer **2**. Paraphaeosphaerin B was thus identified as 3-(8′*R*-hydroxy-5′*R*,6′*R*-oxirenonona-1′*E*,3′*E*-dienyl)-6,8-dihydroxy-isochromen-1-one (**2**).

Paraphaeosphaerin C (**3**), 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 spectral data and indicated nine degrees of unsaturation. ^{1}H and ^{13}C NMR data (Tables 1 and 2, respectively) showed very close resemblance to those of **1** and **2** suggesting that **3** had the same carbon skeleton. However, ^{1}H and ^{13}C NMR spectra of **3** lacked signals due to two olefinic hydrogens/carbons but showed the presence of a methylene carbon (δ_H 1.87; δ_C 38.9) and an oxygenated methine carbon (δ_H 4.45; δ_C 68.9). In the ^{1}H–^{1}H COSY spectrum, the H-1′ at δ 6.22 (d, J=15.5 Hz) showed a correlation with H-2′ at δ 6.52 (dd, J=15.5, 5.0 Hz), which also was found to have a cross peak with 3′-H (δ 4.45); 3′-H also showed correlations with 4′-H at δ 1.70 (m) and 1.87 (dt, J=14.1 and 6.1 Hz) suggesting that C-3′ is oxygenated. Detailed analysis of 1D and 2D NMR spectra permitted the assignment of all proton and carbon signals of paraphaeosphaerin C (Tables 1 and 2). Since there was an additional asymmetric carbon atom in **3** compared with **1** and **2**, the absolute stereochemistry of this was determined by the application of a modified Mosher's method.[10] Reaction of the monomethyl paraphaeosphaerin C (**12**) with (*S*)- and (*R*)-α-methoxy-α-trifluoromethylphenylacetic (MTP) acids afforded (*R*)- and (*S*)-MTPA esters (**13a** and **13b**; Fig. 2), respectively. Analysis of $\Delta\delta$ values (Fig. 2) confirmed the *S* absolute stereochemistry for C-4′ and *R* absolute stereochemistry for C-8′. The structure of paraphaeosphaerin C was thus established as 3-(4′*S*,8′*R*-dihydroxy-5′*R*,6′*R*-oxirenonona-1′*E*-enyl)-6,8-dihydroxy-isochromen-1-one (**3**).

Aposphaerin C (**8**) was determined to have the molecular formula $C_{16}H_{18}O_5$ by HRMS, which was consistent with its ^{13}C and HSQC NMR data and indicated eight degrees of unsaturation. It had IR absorption bands at 3525, 1704, and 1651 cm^{-1} indicating the presence of hydroxyl, carboxylic acid, carbonyl, and conjugated carbonyl functionalities.



13a : R = (*S*)-MTP
13b : R = (*R*)-MTP
14a : R = (*S*)-MTP
14b : R = (*R*)-MTP

Figure 2. $\Delta\delta$ value [($\Delta\delta$ in ppm)=$\delta_S-\delta_R$] obtained for (*S*)- and (*R*)-MTP esters (**13a** and **13b**, respectively) of monomethylparaphaeosphaerin A (**12**) and (**14a** and **14b**, respectively) of chaetochiversin A (**4**).

Its UV spectrum with absorption maxima at 311.5, 277.5, 237.0, and 220.5 nm was characteristic of a 7-hydroxychroman-4-one structure.[11] Comparison of its ^{1}H and ^{13}C NMR data with those reported for aposphaerin B (**15**) suggested that **15** may be the ethyl ester of aposphaerin C (**8**) and this was further confirmed by the application of 2D NMR techniques including ^{1}H–^{1}H COSY and HMBC. Methylation of **8** with CH_3I/K_2CO_3 in acetone yielded its dimethyl derivative **16**. Isolation of aposphaerin B and the related octaketide, cavoxinone[11] as racemic mixtures has led to the suggestion that they have been formed by non-enzymatic cyclizations of appropriate olefinic open chain precursors.[11] The absence of any optical rotation associated with aposphaerin C (**8**) suggested that it may also have been formed by a similar process. The structure of aposphaerin C was thus elucidated as 3,4-dihydro-7-hydroxy-4-oxo-2-(1*E*-pentenyl)-2*H*-1-benzo-pyran-5-acetic acid (**8**).

Initial liquid–liquid partitioning of the EtOAc extract of the endophytic fungus, *C. chiversii*, indicated that the cytotoxicity was concentrated in the 80% aqueous MeOH soluble fraction. Size exclusion chromatography of this fraction on Sephadex LH-20 followed by column chromatography over silica gel and repeated thin layer chromatography furnished radicicol (**7**),[1] two new isocoumarins, chaetochiversins A (**4**) and B (**5**), and the three known chromones **9**–**11**. Chaetochiversin A (**4**) was obtained as a pale yellow solid that was analyzed for $C_{18}H_{17}ClO_6$ by a combination of HRFABMS and ^{13}C NMR spectroscopy. Its IR, UV, and ^{1}H NMR spectra were similar to those of paraphaeosphaerin A (**1**) indicating that it is an isocoumarin with 8′-hydroxy-5′,6′-oxirenonona-1′,3′-dienyl side-chain as a substituent. The ^{1}H NMR spectrum of **4** (Table 1) showed the absence of one of the *meta*-coupled protons suggesting that this may be substituted by a Cl atom. Detailed analysis of the HMBC spectra of **4** as for **1** (Fig. 1) suggested that the side-chain is at C-3 position of the isocoumarin moiety. By analogy with the structure of radicicol (**7**), the Cl atom in **4** was placed at C-5. Treatment of radicicol (**7**) with potassium *tert*-butoxide in DMF[8] as for monocillin I above, yielded **4** confirming its structure and the absolute configuration *R* of the three asymmetric centers. The *R* configuration of the C-8′ of chaetochiversin A was further confirmed by ^{1}H NMR analysis[10,12] of its (*S*)- and (*R*)-MTPA esters (**14a** and **14b**; Fig. 2), respectively. On the basis of the above data, the structure of chaetochiversin A was elucidated as 3-(8′*R*-hydroxy-5′*R*,6′*R*-oxirenonona-1′*E*,3′*Z*-dienyl)-5-chloro-6,8-dihydroxy-isochromen-1-one (**4**). Chaetochiversin B (**5**) was obtained as a pale yellow solid. Its molecular formula was established as $C_{18}H_{17}ClO_6$ by a combination of HRFABMS and ^{13}C NMR spectroscopy. ^{1}H and ^{13}C NMR spectral data of **5** were found to be very similar to those of **4**, except the coupling constant between H-3′ and H-4′ (J=15.3 Hz) suggesting the trans relationship of these protons. Treatment of **4** with iodine yielded **5** confirming its structure and configuration of the three asymmetric centers. Chaetochiversin B was therefore identified as 3-(8′*R*-hydroxy-5′*R*,6′*R*-oxirenonona-1′*E*,3′*E*-dienyl)-5-chloro-6,8-dihydroxy-isochromen-1-one (**5**). Comparison of spectral data of the remaining metabolites of *C. chiversii* with those reported in the literature allowed these to be identified as eugenetin (**9**),[13] 6-methoxymethyleugenin (**10**),[14] and 6-hydroxymethyleugenin (**11**).[14,15] Of the compounds encountered in this study only monocillin I (**6**) and radicicol (**7**) were found to exhibit cytotoxic activity.[1]

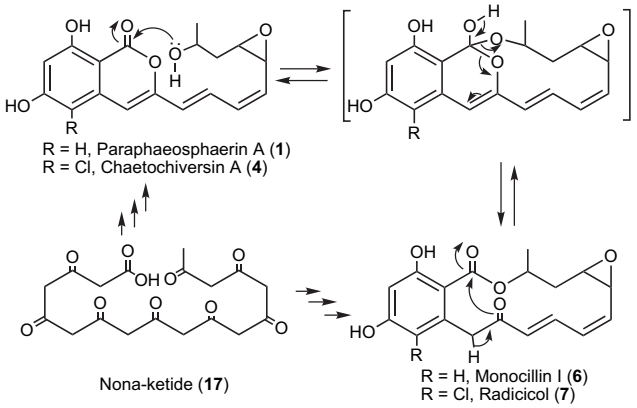


Figure 3. Possible biosynthetic relationship between paraphaeosphaerin A (**1**), monocillin I (**6**), chaetochiversin A (**4**), and radicicol (**7**).

Co-occurrence of the isocoumarins, paraphaeosphaerins A–C (**1**–**3**) and chaetochiversins A and B (**4** and **5**), and the β-resorcylic acid lactone macrolides, monocillin I (**6**) and radicicol (**7**), in these fungal strains suggests that their biosynthesis may involve the common nonaketide precursor (**17**). As depicted in Fig. 3, it may also be possible that the biosyntheses of these macrocyclic lactones may involve the intermediacy of isocoumarins; it is noteworthy that isocoumarins structurally related to paraphaeosphaerins have served as intermediates in recent chemical syntheses of monocillin I.[16] The possibility that these isocoumarins are artifacts arising as a result of the isolation process was ruled out as TLC analysis indicated the presence of these compounds in the original EtOAc extracts and prolonged treatment of monocillin I (**6**) and radicicol (**7**) with MeOH, the solvent used for the extraction of the fungal cultures, failed to yield even trace amounts of paraphaeosphaerin A (**1**) or chaetochiversin A (**4**). The absence of the macrolactone corresponding to paraphaeosphaerin C (**3**) in the EtOAc extract of *P. quadriseptata* further suggests that the isocoumarins encountered in this study are genuine natural products.

3. Experimental

3.1. General experimental procedures

Melting points were determined with an electrothermal melting point apparatus and were uncorrected. Optical rotations were measured with a Jasco Dip-370 digital polarimeter using $CHCl_3$ or MeOH as solvent. UV spectra were recorded on a Shimadzu UV-1601 UV–VIS spectrophotometer. IR spectra for KBr discs were recorded on a Shimadzu FTIR-8300 spectrometer. ^{1}H and 2D NMR spectra were recorded in $CDCl_3$+CD_3OD or acetone-d_6 with a Bruker DRX-600 instrument at 600 MHz for ^{1}H NMR using residual $CHCl_3$ or acetone as internal standard. ^{13}C spectra were recorded with a Bruker DRX-500 instrument at 125 MHz. The chemical shift values (δ) are given in parts per million (ppm), and the coupling constants are in hertz. Low resolution and high resolution MS were recorded, respectively, on Shimadzu LCMS-8000 QPα and JEOL HX110A spectrometers.

3.2. Culturing, extraction, and isolation of metabolites of *P. quadriseptata*

The fungal strain isolated from the rhizosphere of the Christmas cactus (*O. leptocaulis* DC.) growing in Tucson, Arizona was identified by Ms. Donna Bigelow (Division of Plant Pathology, Department of Plant Sciences, University of Arizona) as *P. quadriseptata* by analysis of the ITS regions of the ribosomal DNA as described previously.[5] The strain is deposited in the Department of Plant Pathology and Southwest Center for Natural Products Research and Commercialization of the University of Arizona microbial culture collection under the accession number Opl-1-F20 (AH-45-00-F20). For isolation of bioactive compounds, the fungus was cultured and processed as reported previously[5] to afford the cytotoxic EtOAc extract (1.85 g). A portion (1.80 g) of this extract was partitioned between hexane and 80% aqueous MeOH, and the cytotoxic aqueous MeOH fraction was diluted to 60% aqueous MeOH by the addition of water and extracted with $CHCl_3$. Evaporation of $CHCl_3$ under reduced pressure yielded a pale brown semisolid (1.31 g) that was found to be cytotoxic. A portion (1.30 g) of this was subjected to gel permeation chromatography on a column of Sephadex LH-20 (40.0 g) made up in hexane/CH_2Cl_2 (1:4) and eluted with hexane/CH_2Cl_2 (1:4) (700 mL), CH_2Cl_2/acetone (3:2) (250 mL), CH_2Cl_2/acetone (1:4) (250 mL), and finally with MeOH (250 mL). Twenty-four fractions (50 mL each) were collected (F_1–F_{24}) of which fractions F_7–F_{17} were found to be cytotoxic. These fractions were combined and further fractionated on silica gel (13.0 g) by elution with CH_2Cl_2 followed by increasing amounts of MeOH in CH_2Cl_2. Fractions eluted with 0.5% MeOH in CH_2Cl_2 were found to be cytotoxic and these fractions were combined and evaporated to yield monocillin I (**6**) (521 mg). Chromatography of the fraction F_{17} (114.0 mg) on silica gel (3.0 g) by elution with CH_2Cl_2 followed by increasing amounts of MeOH in CH_2Cl_2 afforded 43 fractions. Of these, the fraction (15.9 mg) eluted with 2% MeOH in CH_2Cl_2 afforded **1** (5.7 mg). Column chromatography of the fraction F_{18} (86.0 mg) on silica gel (3.0 g) by elution with Et_2O followed by Et_2O containing increasing amounts of MeOH afforded several fractions. Of these, the early fractions (4.1 mg) eluted with Et_2O yielded **8** (3.1 mg). Fraction F_{19} (97.0 mg) was further fractionated on silica gel (3.5 g) by elution with increasing amounts of MeOH in CH_2Cl_2 followed by MeOH to give 42 fractions. Of these, the fraction (25.6 mg) eluted with 5% MeOH in CH_2Cl_2 was further separated on preparative TLC on silica gel (CH_2Cl_2/MeOH, 98:2) to give **1** (1.2 mg) and **2** (4.9 mg). Later fractions eluted with 5% MeOH in CH_2Cl_2 were combined and further purified by repeated preparative TLC (silica gel) to give **3** (5.2 mg).

3.2.1. Paraphaeosphaerin A (1). Off-white amorphous solid; mp 178–180 °C; $[\alpha]_D^{25}$ +4.2 (*c* 2.0, MeOH); UV (MeOH) λ_{max} (log ε) 378 (4.23), 360.5 (4.41), 345.5 (4.35), 330 (4.29), 270 (4.66), 208.5 (4.11) nm; IR (KBr) ν_{max} 3380, 1664, 1620, 1569, 1504, 1466, 1366, 1242, 1161, and 1080 cm^{-1}; HRFABMS *m/z* 331.1182 $[M+H]^+$ (calcd for $C_{18}H_{19}O_6$, 331.1182); ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.2.2. Paraphaeosphaerin B (2). Off-white amorphous solid; mp 174–176 °C; $[\alpha]_D^{25}$ +7.2 (*c* 2.0, MeOH); UV (MeOH) λ_{max} (log ε) 378.0 (4.93), 360.5 (5.12), 345.5 (5.05), 329.5 (4.10), 270.0 (5.36), 208.5 (4.51) nm; IR (KBr) ν_{max} 3390, 1663, 1620, 1570, 1508, 1462, 1362, 1242, 1165, 1080, 1038, and 972 cm^{-1}; HRFABMS *m/z* 331.1182 $[M+H]^+$ (calcd for $C_{18}H_{19}O_6$, 331.1182); ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.2.3. Paraphaeosphaerin C (3). Off-white amorphous solid; mp 132–134 °C; $[\alpha]_D^{25}$ −5.8 (*c* 1.0, MeOH); UV (MeOH) λ_{max} (log ε) 354.5 (4.75), 339 (4.32), 312 (4.75), 301 (4.75), 259 (5.47), 203 (4.88) nm; IR (KBr) ν_{max} 3395, 1678, 1628, 1578, 1508, 1462, 1366, 1238, 1169, and 1072 cm^{-1}; HRFABMS *m/z* 349.1287 $[M+H]^+$ (calcd for $C_{18}H_{21}O_7$, 349.1287); ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.2.4. Aposphaerin C (8). White solid; mp 154–156 °C; UV (MeOH) λ_{max} (log ε) 311.5 (2.75), 277.5 (3.09), 237 (3.06), 220.5 (3.24) nm; IR (KBr) ν_{max} 3525, 1651, 1589, 1504, 1454, 1362, 1288, 1157, and 1053 cm^{-1}; ^1H NMR [$^2H_6$]-acetone δ: 6.41 (1H, d, *J*=2.4 Hz, H-6), 6.35 (1H, d, *J*=2.4 Hz, H-8), 5.90 (1H, ddt, *J*=15.6, 6.6, 0.8 Hz, H-2′), 5.69 (1H, ddt, *J*=15.6, 6.6, 1.3 Hz, H-1′), 4.89 (1H, m, H-2), 3.96 (1H, d, *J*=16.6 Hz, H-1″a), 3.89 (1H, d, *J*=16.6 Hz, H-1″b), 2.69 (1H, dd, *J*=16.5, 11.8 Hz, H-3a), 2.55 (1H, dd, *J*=16.5, 3.3 Hz, H-3b), 2.07 (2H, br t, *J*=7.2 Hz, H_2-3′), 1.42 (2H, dq, *J*=7.2, 7.2 Hz, H_2-4′), 0.91 (3H, t, *J*=7.2 Hz, CH_3-5′); ^{13}C NMR [$^2H_6$]-acetone δ: 192.4 (C-2″), 172.5 (C-4), 165.8/164.2 (C-7/C-8a), 141.1 (C-5), 135.6 (C-2′), 129.5 (C-1′), 115.5 (C-6), 114.3 (C-4a), 103.6 (C-8), 79.1 (C-2), 45.1 (C-3), 41.6 (C-1″), 35.4 (C-3′), 23.3 (C-4′), 14.4 (C-5′); HRFABMS *m/z* 291.1232 $[M+H]^+$ (calcd for $C_{16}H_{19}O_5$, 291.1232).

3.2.5. Conversion of monocillin I (6) to 1. To a solution of **6** (20 mg) in DMF (500 μL) was added a solution of *t*-BuOK (33 mg) in *t*-BuOH (200 μL) and warmed at 50 °C for 10 min and allowed to stand at 25 °C for 40 min. Water (5 mL) was added to the reaction mixture, acidified with 2 N HCl, and extracted with EtOAc (3×15 mL). Purification by preparative TLC (silica gel) using 10% MeOH in CH_2Cl_2 as eluant gave **1** (1.2 mg).

3.2.6. Isomerization of paraphaeosphaerin A (1) to paraphaeosphaerin B (2). A solution of iodine (0.2 mg) in EtOAc (100 μL) was added to a solution of **1** (1.0 mg) in EtOAc (100 μL) and stirred at 25 °C. After 5 min MeOH (100 μL) was added to the reaction mixture, solvents removed under reduced pressure, and the crude product was purified by preparative TLC (silica gel) using 10% MeOH in CH_2Cl_2 as eluant to give **2** (0.8 mg).

3.2.7. Monomethyl paraphaeosphaerin C (12). K_2CO_3 (20 mg) and CH_3I (200 μL) were added to a solution of **3** (2.0 mg) in acetone (400 μL) and stirred at 25 °C for 4 h after which the reaction mixture was filtered and the filtrate evaporated under reduced pressure. The crude product was purified by preparative TLC (silica gel) using 8% MeOH in CH_2Cl_2 as eluant to give **12** as a colorless semisolid (2.1 mg); ^1H NMR acetone-d_6 δ: 11.09 (1H, s, OH), 6.63 (1H, dd, *J*=15.5, 5.0 Hz, H-2′), 6.62 (1H, s, H-4), 6.60 (1H, d, *J*=2.0 Hz, H-5), 6.49 (1H, d, *J*=2.0 Hz, H-7), 6.43 (1H, dd, *J*=15.5, 1.5 Hz, H-1′), 4.55 (1H, dd, *J*=6.0,

5.0 Hz, H-3′), 3.96 (1H, m, H-8′), 3.92 (3H, s, OMe), 2.88 (1H, dt, J=6.0, 2.0 Hz, H-6′), 2.85 (1H, dt, J=6.0, 2.0 Hz, H-5′), 1.91 (1H, dt, J=13.5, 6.0 Hz, H-4′a), 1.77 (1H, dt, J=13.5, 6.0 Hz, H-4′b), 1.73 (1H, dt, J=13.5, 6.0 Hz, H-7′a), 1.52 (1H, dt, J=13.5, 6.0 Hz, H-7′b), 1.18 (3H, d, J=6.3 Hz, CH_3).

3.2.8. Preparation of the (*R*)- and (*S*)-MTPA esters of 12. Monomethyl paraphaeosphaerin A (**12**) (1.0 mg, 2.76 μmol) in anhydrous EtOAc (100 μL) was added to a stirred solution of (*R*)-α-methoxy-α-trifluoromethylphenyl acetic acid (3.0 mg, 12.82 μmol), DCC (3.3 mg, 16.02 μmol), and 4-PP (catalytic amount) in anhydrous EtOAc (150 μL) and stirred at 25 °C. After 4 h (TLC control) the reaction mixture was filtered through a cotton plug and EtOAc was removed. Resulting residue was purified by preparative TLC (silica gel) using 1% methanol in CH_2Cl_2 as eluant to give (*R*)-MTPA ester (**13a**) (1.6 mg). The process was repeated as above, but using (*S*)-α-methoxy-α-trifluoromethylphenyl acetic acid, to afford the (*S*)-MTPA ester (**13a**) (1.5 mg).

*(R)-MTPA ester of **12*** (**13a**): White semisolid; selected ^{1}H NMR signals ($CDCl_3$, 600 MHz) δ: 11.0 (1H, s, OH), 6.48 (1H, d, J=2.1 Hz, H-5), 6.41 (1H, dd, J=15.5, 6.7 Hz, H-2′), 6.36 (1H, d, J=2.1 Hz, H-7), 6.17 (1H, s, H-4), 6.09 (1H, d, J=15.5 Hz, H-1′), 5.75 (1H, dd, J=13.1, 6.7 Hz, H-3′), 5.27 (1H, m, H-8′), 2.72 (1H, dd, J=6.1, 3.8 Hz, H-5′), 2.69 (1H, m, H-6′), 2.02 (1H, m, H-4′a), 1.85 (1H, m, H-7′a), 1.82 (1H, m, H-4′b), 1.81 (1H, m, H-7′b), 1.30 (3H, d, J=6.4 Hz, CH_3).

*(S)-MTPA ester of **12*** (**13b**): White semisolid; selected ^{1}H NMR signals ($CDCl_3$, 600 MHz) δ: 11.03 (1H, s, OH), 6.49 (1H, d, J=2.1 Hz, H-5), 6.49 (1H, dd, J=13.2, 7.4 Hz, H-2′), 6.37 (1H, d, J=2.1 Hz, H-7), 6.29 (1H, s, H-4), 6.27 (1H, d, J=13.2 Hz, H-1′), 5.69 (1H, dd, J=12.2, 6.2 Hz, H-3′), 5.23 (1H, m, H-8′), 2.54 (1H, m, H-5′), 2.53 (1H, m, H-6′), 1.90 (1H, m, H-4′a), 1.80 (1H, m, H-7′a), 1.75 (1H, m, H-7′b), 1.73 (1H, m, H-4′b), 1.37 (3H, d, J=6.4 Hz, CH_3).

3.2.9. Methyl aposphaerin C (16). K_2CO_3 (10 mg) and CH_3I (100 μL) were added to a solution of aposphaerin C (**8**) (1.0 mg) in acetone (200 μL) and stirred at 25 °C for 14 h. Reaction mixture was then filtered and the filtrate evaporated under reduced pressure to give **16** as a white semisolid (1.0 mg); ^{1}H NMR ($CDCl_3$) δ: 6.41 (1H, d, J=2.1 Hz, H-6), 6.34 (1H, d, J=2.1 Hz, H-8), 5.84 (1H, dt, J=15.5, 6.7 Hz, H-2′), 5.63 (1H, dd, J=15.5, 6.8 Hz, H-1′), 4.85 (1H, m, H-2), 3.96 (1H, d, J=16.6 Hz, H-1″a), 3.89 (1H, d, J=16.6 Hz, H-1″b), 3.79 (3H, s, OMe), 3.69 (3H, s, OMe), 2.73 (1H, dd, J=16.5, 12.4 Hz, H-3a), 2.59 (1H, dd, J=16.5, 3.1 Hz, H-3b), 2.05 (2H, br t, J=7.2 Hz, H_2-3′), 1.42 (2H, dq, J=7.3, 7.3 Hz, H_2-4′), 0.90 (3H, t, J=7.3 Hz, CH_3-5′); MS m/z 319 ($[M+H]^+$, in +APCI mode), 317 ($[M-H]^-$, in −APCI mode).

3.3. Culturing, extraction, and isolation of metabolites of *C. chiversii*

The fungal strain was isolated from the stem of *E. fasciculata* growing in South mountain park in Phoenix, Arizona, and was identified by Microbial ID Inc, Newark, DE as *C. chiversii*. The strain is deposited in the School of Life Sciences, Arizona State University, and the Southwest Center for Natural Products Research and Commercialization of the University of Arizona microbial culture collection under the accession numbers 7-EPH-2S and CS-36-62, respectively. For isolation of secondary metabolites, the fungus was cultured and processed as described previously[1] to obtain the EtOAc extract (2.01 g). A portion (1.75 g) of this extract was partitioned between hexane and 80% aqueous MeOH. Evaporation of solvents under reduced pressure yielded hexane (0.535 g) and 80% aqueous MeOH (1.031 g) fractions. A portion (1.0 g) of the 80% aqueous MeOH fraction was subjected to gel permeation chromatography on a column of Sephadex LH-20 (30 g) in hexane/CH_2Cl_2 (1:4) and eluted with hexane/CH_2Cl_2 (1:4) (800 mL), CH_2Cl_2/acetone (3:2) (500 mL), CH_2Cl_2/acetone (1:4) (300 mL), CH_2Cl_2/MeOH (1:1) (300 mL), and finally with MeOH (500 mL). Seventy fractions (20 mL each) were collected and pooled based on their TLC patterns to yield 20 combined fractions (F_1–F_{20}). Fraction 3 (F_3) was chromatographed over a column of silica gel (3.0 g) made up in CH_2Cl_2/hexane (3:2) and eluted with CH_2Cl_2/hexane (3:2 and 3:1), CH_2Cl_2 followed by CH_2Cl_2 containing increasing amounts of MeOH. Sixty fractions were collected and fractions having similar TLC patterns were combined to give six sub-fractions (A–F). Sub-fraction A was separated on preparative TLC (silica gel) using 1% MeOH in CH_2Cl_2 as eluant to give **9** (2.8 mg). Sub-fraction E was separated on preparative TLC (silica gel) using 3% MeOH in CH_2Cl_2 to give **10** (1.2 mg). Fraction F_5 was purified by preparative TLC (silica gel) using 6% MeOH in CH_2Cl_2 as eluant to give **11** (2.8 mg). Fraction F_{12} (250 mg) was chromatographed over a column of silica gel (Fluka G 60, 6.0 g) made up in CH_2Cl_2 and eluted with CH_2Cl_2 containing increasing amounts of MeOH. Eighty fractions (4 mL each) were collected and fractions having similar TLC patterns were combined to give eight sub-fractions. The sub-fraction eluted with 0.5% MeOH in CH_2Cl_2 was washed with hexane/CH_2Cl_2 (2:3) to give **7** (102.0 mg). Fractions F_{14} and F_{15} were combined and chromatographed over a column of silica gel (2.5 g) made up in CH_2Cl_2 and eluted with CH_2Cl_2 followed by CH_2Cl_2 containing increasing amounts of MeOH. Sixty-two fractions (7.5 mL each) were collected and fractions having similar TLC behavior were combined to give 16 sub-fractions. Sub-fraction eluted with 2% MeOH in CH_2Cl_2 was further separated by preparative RP-TLC (RP-18) using 25% H_2O in MeOH as eluant to give **4** (1.3 mg). Preparative TLC (RP-18, 25% H_2O in MeOH) purification of another sub-fraction eluted with 2% MeOH in CH_2Cl_2 gave **5** (1.1 mg).

3.3.1. Chaetochiversin A (4). Pale yellow amorphous solid; mp 186–188 °C; $[\alpha]_D^{23}$ +5.3 (*c* 1.0, MeOH); UV (EtOH) λ_{max} (log ε) 392 (4.85), 374 (5.10), 357 (5.10), 340 (5.07), 326 (5.08), 312 (5.02), 292 (5.35), 273 (5.39); IR (KBr) ν_{max} 3390, 1681, 1616, 1566, 1461, 1392, 1357, 1238, 1184, 1076, and 988 cm^{-1}; HRFABMS m/z $[M+H]^+$ 365.7908 (calcd for $C_{18}H_{18}ClO_6$, 365.7903); ^{1}H and ^{13}C NMR data, see Tables 1 and 2.

3.3.2. Chaetochiversin B (5). Pale yellow amorphous solid; mp 179–181 °C; $[\alpha]_D^{23}$ +8.6 (*c* 1.0, MeOH); UV (EtOH) λ_{max}

(log ε) 392 (4.75), 373 (5.00), 357 (5.01), 340 (4.99), 326 (4.99), 292 (5.29), 274 (5.30); IR (KBr) ν_{max} 3387, 1679, 1618, 1569, 1463, 1390, 1360, 1240, 1184, and 1076 cm^{-1}; HRFABMS *m/z* $[M+H]^+$ 365.7908 (calcd for $C_{18}H_{18}ClO_6$, 365.7903); ^1H and ^{13}C NMR data, see Tables 1 and 2.

3.3.3. Eugenetin (9). White crystalline solid; mp 160–162 °C (lit.[13] 159–160 °C); ^1H NMR data were consistent with literature values;[13] APCIMS (+)ve mode *m/z* 221 $[M+H]^+$.

3.3.4. 6-Methoxymethyleugenin (10). White crystalline solid; mp 192–193 °C; ^1H NMR data were consistent with literature values;[14] APCIMS (+)ve mode *m/z* 219 $[M+H-MeOH]^+$.

3.3.5. 6-Hydroxymethyleugenin (11). White crystalline solid; mp 197–198 °C (lit.[15] 198–199 °C); ^1H NMR data were consistent with literature values;[13] APCIMS (+)ve mode *m/z* 219 $[M+H-H_2O]^+$.

3.3.6. Conversion of radicicol (7) to chaetochiversin A (4). To a solution of **7** (10.0 mg) in DMF (0.5 mL) was added *t*-BuOK (11.3 mg). The reaction mixture was warmed to 50 °C and stirred for 3 h, after which it was poured into brine and extracted with EtOAc (3×30 mL). EtOAc extracts were combined, washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure and the crude product separated on preparative TLC (silica gel) using 6% MeOH in CH_2Cl_2 as eluant to give **4** (5.0 mg) and unreacted radicicol (**7**) (1.2 mg).

3.3.7. Preparation of the (*R*)- and (*S*)-MTPA ester derivatives of chaetochiversin A. Chaetochiversin A (**4**, 0.5 mg) was transferred into a clean NMR tube and was dried completely under the vacuum of an oil pump. Deuterated pyridine (0.6 mL) and (*R*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride (5.0 μL) were added into the NMR tube immediately under a stream of N_2, and then the NMR tube was shaken carefully to mix the sample and MTPA chloride evenly. The reaction NMR tube was permitted to stand at room temperature for 8 h. ^1H NMR data of the (*R*)-MTPA ester derivative (**14a**) of **7** (500 MHz, pyridine-d_5) δ: 6.690 (1H, dd, *J*=15.2, 10.1 Hz, H-2′), 6.832 (1H, s, H-4), 6.660 (1H, s, H-7), 6.357 (1H, d, *J*=15.2 Hz, H-1′), 6.262 (1H, dd, *J*=11.7, 10.6 Hz, H-3′), 6.115 (1H, dd, *J*=15.2, 6.3 Hz, H-4′), 5.813 (1H, m, H-8′), 4.864 (1H, m, H-5′), 4.436 (1H, m, H-6′), 2.298 (1H, dd, *J*=13.1, 5.2 Hz, H-7′a), 1.897 (1H, ddd, *J*=13.1, 9.4, 4.7 Hz, H-7′b), 1.189 (1H, d, *J*=6.1 Hz, CH_3-8′); APCIMS (+)ve mode *m/z* 625 $[M+H]^+$. In the manner described for **14a** another portion of **4** (0.5 mg) was reacted in a second NMR tube with (*S*)-(−)-α-methoxy-α-trifluoromethylphenylacetyl (MTPA) chloride (5.0 μL) at room temperature for 8 h using pyridine-d_5 (0.6 mL) as solvent, to afford the (*S*)-MTPA ester (**14b**). ^1H NMR data of **14b** (500 MHz, pyridine-d_5) δ: 6.952 (1H, dd, *J*=15.2, 11.8 Hz, H-2′), 6.821 (1H, s, H-4), 6.675 (1H, s, H-7), 6.562 (1H, dd, *J*=15.2, 10.8 Hz, H-3′), 6.265 (1H, d, *J*=15.2 Hz, H-1′), 5.840 (1H, m, H-8′), 5.765 (1H, dd, *J*=15.2, 6.4 Hz, H-4′), 4.808 (1H, dd, *J*=6.4, 3.4 Hz, H-5′), 4.425 (1H, m, H-6′), 2.215 (1H, dd, *J*=13.1, 5.2 Hz, H-7′a), 1.877 (1H, ddd, *J*=13.1, 9.4, 4.7 Hz, H-7′b), 1.216 (1H, d, *J*=6.1 Hz, CH_3-8′); APCIMS (+)ve mode *m/z* 625 $[M+H]^+$.

3.3.8. Conversion of chaetochiversin A (4) to chaetochiversin B (5). A solution of iodine (0.2 mg) in EtOAc (100 μL) was added to a solution of **4** (1.0 mg) in EtOAc (100 μL) and stirred at 25 °C. After 5 min (TLC control), MeOH (100 μL) was added to the reaction mixture, solvents removed under reduced pressure and the crude product was purified by preparative TLC (silica gel) using 8% MeOH in CH_2Cl_2 as eluant to afford **5** as a pale yellow solid (0.9 mg).

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