



# Five new isocoumarins from Sonoran desert plant-associated fungal strains *Paraphaeosphaeria quadrisepitata* and *Chaetomium chiversii*<sup>☆</sup>

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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 quadrisepitata* 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. quadrisepitata*. Their structures and stereochemistry were elucidated using a combination of <sup>1</sup>H and <sup>13</sup>C homo- and hetero-nuclear 2D NMR techniques, <sup>1</sup>H NMR analysis of Mosher's esters, and chemical correlations.  
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## 1. Introduction

Recent studies have demonstrated that plant-associated fungi are rich sources of structurally diverse natural products, some with interesting biological activities.<sup>2</sup> 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 quadrisepitata* 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-methoxymethyleugenin (**10**), and 6-hydroxy-methyleugenin (**11**). Previous studies of *P. quadrisepitata* and *C. chiversii* have resulted in the isolation of cytotoxic and heat shock protein-90 (Hsp90) inhibitory  $\beta$ -resorcylic acid lactone macrolides, monocillin I (**6**) and radicicol (**7**), respectively.<sup>1</sup> Isolation of two 10-membered macrolides, modiolides A and B, from the marine-derived *Paraphaeosphaeria* sp. N-119 has recently been reported.<sup>3</sup>

## 2. Results and discussion

Liquid–liquid partitioning<sup>4</sup> of the EtOAc extract of a solid culture of *P. quadrisepitata* followed by size exclusion chromatography of the CHCl<sub>3</sub> soluble fraction on Sephadex LH-20 and chromatographic separation of the fraction eluted with hexane/CH<sub>2</sub>Cl<sub>2</sub> (1:4) and CH<sub>2</sub>Cl<sub>2</sub>/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**).<sup>5</sup>

Paraphaeosphaerin A (**1**) was obtained as a white amorphous solid that was analyzed for C<sub>18</sub>H<sub>18</sub>O<sub>6</sub> by a combination of HRFABMS and <sup>13</sup>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<sup>-1</sup> suggested the presence of OH/NH,  $\alpha,\beta$ -unsaturated lactone carbonyl and olefinic groups. In the <sup>1</sup>H NMR spectrum of **1** (Table 1), in addition to other signals, a chelated OH ( $\delta$  11.12), a set of *meta*-coupled one-proton doublets [ $\delta$  6.24 and 6.32 ( $J=2.0$  Hz)], and five olefinic/aromatic protons [ $\delta$  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 <sup>13</sup>C NMR spectrum of **1** (Table 2) indicated the presence of an  $\alpha,\beta$ -unsaturated lactone/ester carbonyl, three oxygenated and nine non-oxygenated olefinic/aromatic carbons. In the HMBC spectrum, the proton at  $\delta$  6.22 (H-4) showed a correlation with a quaternary carbon at  $\delta$  99.9 (C-8a) and an aromatic carbon at  $\delta$  104.4 (C-5) bearing one of the *meta*-coupled protons [ $\delta$  6.32 (H-5)].

\* See Ref. 1.

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

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