Heptaketides from *Corynespora* sp. Inhabiting the Cavern Beard Lichen, *Usnea cavernosa*: First Report of Metabolites of an Endolichenic Fungus[1]

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Two new heptaketides, corynesporol (**1**) and 1-hydroxydehydroherbarin (**2**), along with herbarin (**3**) were isolated from an endolichenic fungal strain, *Corynespora* sp. BA-10763, occurring in the cavern beard lichen *Usnea cavernosa*. The structures of **1**−**3** were elucidated from their spectroscopic data. Aerial oxidation of corynesporol (**1**) yielded herbarin (**3**). Acetylation of **1** afforded the naphthalene derivative **4**, whereas acetylation of **3** gave the corresponding naphthoquinone **6** and dehydroherbarin (**5**). All compounds were evaluated for their cytotoxicity and ability to inhibit migration of human metastatic breast and prostate cancer cell lines MDA-MB-231 and PC-3M, respectively. Dehydroherbarin (**5**) inhibited migration of both cell lines at concentrations not toxic to these cell lines. This is the first report of metabolites from an endolichenic fungus.

Lichens are symbiotic associations of algae or cyanobacteria (photobiont) and filamentous fungi (mycobiont). The photobiont provides photosynthate to the mycobiont, which in turn makes up the bulk of the lichen thallus and provides the alga with access to water and minerals. Mycobionts also frequently produce secondary metabolites to protect lichens against harmful radiation, herbivores, and microbial infections.[2] Although viewed traditionally as bipartite or tripartite symbioses, lichens frequently harbor diverse fungi in addition to the fungal mycobiont. Incidental fungi on thallus surfaces, as well as lichenicolous fungi (relatively obligate associates that fruit from lichen thalli), have been recognized for some time,[3] and recent work has shown that lichens also harbor numerous endolichenic fungi, fungi that live within the asymptomatic lichen thallus much the same way as endophytic fungi live within healthy plant tissues.[4] For example, 1086 fungal strains have been isolated from the exterior and interior of the lichen *Letharietum vulpinae* (Parmeliaceae; Lecanorales).[5] Similarly, isolates of 640 endolichenic fungi were recovered from the lichen *Peltigera neopolydactyla* (Peltigeraceae; Peltigerales), in sites ranging from tropical rainforests to Arctic tundra, suggesting that endolichenic fungi are at least as diverse as endophytic fungal communities and, at higher latitudes, are more abundant than endophytes given the same sampling effort.[6] These findings, combined with the occurrence of over 600 species of lichens belonging to over 140 genera in the Sonoran Desert,[7] provide an opportunity of harvesting numerous endolichenic fungi from this biogeographic region.

In a continuation of our studies on arid land plants and microorganisms,[1,8] we have investigated an endolichenic fungal strain collected in the Sonoran desert of the U.S. Southwest. Although a number of lichens,[9] their fungal mycobionts,[10] and a lichenicolous fungus[11] from different geographic locations have been subjected to chemical investigations, leading to the isolation and characterization of a variety of metabolites, to the best our knowledge this constitutes the first report of secondary metabolites from an endolichenic fungus. The endolichenic fungus used in this study, *Corynespora* sp. BA-10763, was isolated from the lichen *Usnea cavernosa* (cavern beard lichen; Parmeliaceae; Lecanorales), collected in early 2005 from Coronado National Forest, Arizona. An EtOAc extract of *Corynespora* sp. BA-10763 cultured in potato dextrose broth (PDB) exhibited significant cell migration inhibitory activity in a wound-healing assay (WHA)[8] using the metastatic prostate cancer cell line, PC-3M, at a noncytotoxic concentration of 5 μg/mL. Herein we report the bioassay-guided isolation of two new heptaketides, corynesporol (**1**) and 1-hydroxydehydroherbarin (**2**), together with herbarin (**3**), the chemical transformation of **1**−**3** yielding compounds **4**−**9**, and evaluation of all compounds for their PC-3M and MDA-MB-231 (metastatic human breast cancer) cell migration inhibitory activities. Herbarin (**3**) and dehydroherbarin (**5**), with weak antimicrobial and antiamebic activities, have previously been reported from the dematiaceous fungus *Torula herbarum*.[12]

Results and Discussion

Bioassay-guided fractionation of a WHA-active EtOAc extract of a liquid culture supernatant of *Corynespora* sp. BA-10763, involving normal-phase and reversed-phase column chromatography and preparative TLC, furnished **1**−**3**. Corynesporol (**1**), isolated as a white solid, was determined to have the molecular formula $C_{16}H_{20}O_6$ by a combination of HRFABMS and ^{13}C and DEPT NMR data and indicated seven degrees of unsaturation. Its IR spectrum with absorption bands at 3413 and 1681 cm^{-1} suggested the presence of OH and conjugated carbonyl groups. The ^1H NMR spectrum of **1** when analyzed with the help of DQF-COSY indicated the presence of two spin systems in addition to three 3H singlets due to two OCH_3 groups (δ 3.96 and 3.89) and a CH_3 (δ 1.42) on a quaternary carbon. The spin system consisting of two 1H doublets was shown to be due to *meta*-coupled protons [δ 7.07 and 6.90 (J = 2.2 Hz)]. The second spin system was found to be complex and consisted of two ABX spin systems connected to each other. One of these spin systems in **1** contained three signals centered at δ 4.14 (1H, t, J = 11.0 Hz), 3.91 (1H, dd, J = 11.0 and 4.5 Hz), and 2.95 (1H, ddd, J = 13.0, 11.0, and 4.5 Hz). The chemical shifts and coupling constants of the A and B portions of this spin system were indicative of nonequivalent methylene protons of a carbon bearing an oxygen atom. The second ABX spin system in **1** contained three signals centered at δ 2.22 (1H, dd, J = 13.0 and 3.5 Hz), 1.62 (1H, dd, J = 13.0 and 11.0 Hz), and 3.35 (1H, ddd, J = 13.0, 11.0, and 3.5 Hz). The DQF-COSY spectrum of **1** also

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