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Isocoumarins and Dihydroisocoumarins From the Endophytic Fungus *Biscogniauxia capnodes* Isolated From the Fruits of *Averrhoa carambola*

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Abstract

An endophytic fungus *Biscogniauxia capnodes* was isolated from a popular edible fruit *Averrhoa carambola*. The fungus was fermented in potato dextrose broth for 3 weeks, and then the culture broth and mycelium were extracted with ethyl acetate. Chromatographic separation of this extract furnished 2 isocoumarins, reticulol (**1**) and 6-*O*-methyl-reticulol (**2**), and 2 dihydroisocoumarins, 5-methylmellein (**3**) and 7-hydroxy-5-methylmellein (**4**). Compound **1** showed moderate antioxidant activity against 2,2′-diphenyl-1-picrylhydrazyl radicals (IC_{50} value, 58 µg/mL). This is the first report of the isolation of *B. capnodes* as an endophyte, as well as the compounds **1** to **4** from *B. capnodes*.

Keywords

endophytic fungi, *Biscogniauxia capnodes*, *Averrhoa carambola*, isocoumarin, dihydroisocoumarin

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Endophytes, microorganisms that reside in the tissues of living plants, have been attracting a growing interest as potential sources of novel natural products for exploitation in medicine, agriculture, and industry. In a continuation of our search for bioactive compounds from endophytic fungi from Sri Lankan flora,[1] we have investigated the secondary metabolites produced by an endophytic fungus, *Biscogniauxia capnodes* (Family: Xylariaceae), isolated from the fruits of *Averrhoa carambola* L. (Oxalidaceae), commonly called star fruit. A variety of carotenoid-derived compounds have been reported from star fruit.[2] We previously reported the chemistry and phytotoxic activity of the constituents of *A. carambola* fruits.[3]

An endophytic fungus isolated from *A. carambola* fruits was identified as *B. capnodes* by morphological studies and sequence analysis of internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA). This is the first report of the isolation of *B. capnodes* from *A. carambola* fruits. *Biscogniauxia capnodes* was cultured in potato dextrose broth (PDB). The ethyl acetate (EtOAc) extract of the broth and mycelium showed antifungal activity against *Cladosporium cladosporioides*, antioxidant activity against 2,2′-diphenyl-1-picrylhydrazyl (DPPH) radicals (42.5% inhibition at 25 ppm), high brine shrimp lethality (96.7% at 125 ppm), and phytotoxicity in the lettuce (*Lactuca sativa*) seed germination assay (shoot inhibition 45.3% and root inhibition 55.3% at 200 ppm). Chromatographic separation of the EtOAc extract furnished two isocoumarins, reticulol (**1**)[4] and 6-*O*-methylreticulol (**2**),[5] and 2 dihydroisocoumarins, 5-methylmellein (**3**)[6] and 7-hydroxy-5-methylmellein (**4**) (Figure 1).[7] The structures of compounds **1** to **4** were elucidated by the analysis of nuclear magnetic resonance (NMR) spectroscopic data and by comparison with reported data, as well as mass spectrometry (MS) data (Table S1). Among these secondary metabolites, compound **3** was most abundant. Compounds **3** and **4** were found to have (3*R*)-absolute configuration on the basis of their negative optical rotations (**3**: $[\alpha]_D^{25}$ −98.9 (*c*, 1.87, $CHCl_3$) (lit. $[\alpha]_D$ −90 ($CHCl_3$)[8] and **4**: $[\alpha]_D^{25}$ −88.9 (*c*, 0.18, $CHCl_3$) (lit. $[\alpha]_D$ −57.4 ($CHCl_3$).[7] It seems likely that the fungi biosynthesize

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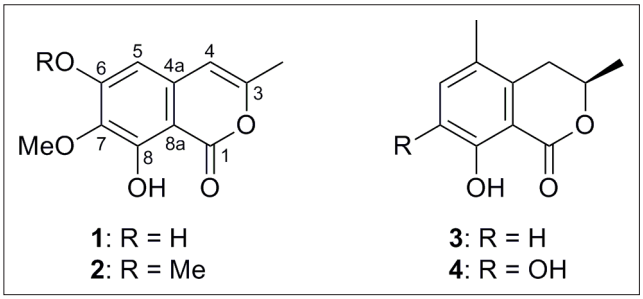

Figure 1. Structures of compounds **1** to **4**.

preferentially (3*R*)-enantiomers of **3** and **4**, since the (3*S*)-antipodes have not been reported from natural sources thus far. Compounds **1** to **4** were subjected to the above-mentioned bioassays, but no significant activities were observed except for reticulol (**1**), which exhibited a moderate DPPH radical scavenging activity with an IC_{50} value of 58 µg/mL (IC_{50} of a positive control butylated hydroxyanisole was 5.5 µg/mL).

Compounds **1** to **4**, all possessing an isocoumarin skeleton, are rather common fungal metabolites, except for compound **4**. Reticulol (**1**) has been reported as an inhibitor of cyclic adenosine 3′,5′-monophosphate phosphodiesterase.[9] Further, reticulol was suggested to inactivate Topo I, which is involved in tumor metastasis, and exhibit excellent cytotoxicity against B16F10 melanoma when combined with adriamycin.[10] Recently, reticulol has been reported to decrease degranulation and histamine release significantly.[11] 6-*O*-Methylreticulol (**2**) was previously isolated from *Streptomyces mobaraensis*[12] and the liverwort *Wettsteinia schusterana*.[13] α-Glucosidase inhibitory activity of **2** has been reported recently.[14] 5-Methylmellein (**3**), isolated from the endophytic fungus *Biscogniauxia mediterranea* that was separated from the medicinal cactus *Opuntia humifusa* was shown to have antifungal activity against the plant pathogen *Phomopsis obscurans*.[6] 7-Hydroxy-5-methylmellein (**4**) was previously reported from the marine-derived fungi *Acremonium* sp. and *Nodulisporium* sp.[7] This paper represents the second isolation of this rare dihydroisocoumarin.

Experimental

General

^1H and ^{13}C NMR were recorded on a JEOL JMN-AL300 (300 MHz for ^1H and 75 MHz for ^{13}C) spectrometer (JEOL, Tokyo, Japan) in $CDCl_3$ solution. Fast atom bombardment mass spectra were obtained on a JEOL JMS-700 spectrometer (JEOL, Tokyo, Japan), while electrospray ionization mass spectra were recorded on Hitachi Chromaster 5610 MS detector (Hitachi, Tokyo, Japan).

Isolation and Identification of the Endophytic Fungus

Healthy, matured, and ripened fruits of *A. carambola* were collected from a home garden in Kandy in 2014. The fruits were rinsed gently in running tap water and triple sterilized with 2% sodium hypochlorite (NaOCl) and 75% ethanol and finally washed with sterile distilled water. A few segments of the inner fleshy part of the fruit were placed on potato dextrose agar medium and allowed to stand at room temperature for 2 to 3 days in the dark. The emerging fungus was subcultured serially to obtain a pure culture of the fungus, which was identified as *B. capnodes* by sequence analysis of ITS1 and ITS4 regions of the rDNA gene. Search using Basic Local Alignment Search Tool indicated that the sequence of the ITS region matched 95% that of *B. capnodes* (Gene Bank Acc. No. EF026131.1). Fungal identification was carried out by GeneTech Institute, Colombo. Photographic evidence of the fruits of *A. carambola* and *B. capnodes* strain (TAC-2014) is deposited at the National Institute of Fundamental Studies.

Fermentation of Fungus, Extraction, Isolation of Compounds, and Bioassays

Biscogniauxia capnodes was cultured by inoculating flasks containing 400 mL of PDB medium. The flasks were allowed to stand at room temperature for 10 days, then shaken every other day on a laboratory shaker. After 21 days, the culture media were filtered and the filtrate extracted with EtOAc (×3). The residual mycelium was extracted with EtOAc using a sonicator. Based on the thin-layer chromatography (TLC) analysis both EtOAc extracts were combined. The combined extract was screened for antifungal activity against *C. cladosporioides* by a TLC bioautography method,[15] DPPH radical scavenging activity with a spectrophotometric method,[16] brine shrimp (*Artemia salina*) lethality,[17] phytotoxicity using lettuce seed germination assay,[18] and α-amylase inhibitory activity using a spectrophotometric method.[19] Chromatographic separation of the EtOAc extract (1.46 g) over silica gel, Sephadex LH-20, and preparative TLC furnished reticulol (**1**, 5 mg), 6-*O*-methylreticulol (**2**, 11 mg), 5-methylmellein (**3**, 36 mg) and 7-hydroxy-5-methylmellein (**4**, 4 mg).

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Supplemental Material

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References

1. Padmathilake KGE, Bandara HMSKH, Qader MM, et al. Talarofuranone, a new talaroconvolutin analog from the endophytic fungus *Talaromyces purpurogenus* from *Pouteria campechiana* Seeds. *Nat Prod Commun*. 2017;12(4):489-490.
2. Winterhalter P, Lutz A, Herderich M, Schreier P. Starfruit (Averrhoa carambola L.). Attractive source for carotenoid-derived flavor compounds. *ACS Symp Ser*. 1995;596:114-126.
3. Gunawardena DC, Jayasinghe L, Fujimoto Y. Phytotoxic constituents of the fruits of *Averrhoa carambola*. *Chem Nat Compd*. 2015;51(3):532-533.
4. Furutani Y, Tsuchiya I, Naganawa H, Takeuchi T, Umezawa H. Biosynthetic studies of reticulol, an isocoumarin, by ^{13}C-NMR spectroscopy. *Agri Biol Chem*. 1977;41(9):1581-1585.
5. Saeed A, Ehsan S. An efficient synthesis of 8-Hydroxy-6,7-dimethoxy-3-methylisocoumarin (6-*O*-methylreticulol). *Chem Heterocycl Compd*. 2005;41(11):1381-1385.
6. Silva-Hughes AF, Wedge DE, Cantrell CL, et al. Diversity and antifungal activity of the endophytic fungi associated with the native medicinal cactus *Opuntia humifusa* (Cactaceae) from the United States. *Microbiol Res*. 2015;175:67-77.
7. Pontius A, Mohamed I, Krick A, Kehraus S, König GM. Aromatic polyketides from marine algicolous fungi. *J Nat Prod*. 2008;71(2):272-274.
8. Sorres J, Nirma C, Touré S, Eparvier V, Stien D. Two new isopimarane diterpenoids from the endophytic fungus *Xylaria* sp. SNB-GTC2501. *Tetrahedron Lett*. 2015;56(31):4596-4598.
9. Furutani Y, Shimada M, Hamada M, Takeuchi T, Umezawa H. Reticulol, an inhibitor of cyclic nucleotide phosphodiesterases. *Agric Biol Chem*. 1977;41(6):989-993.
10. Lim D-S, Kwak Y-S, Kim J-H, Ko S-H, Yoon W-H, Kim C-H. Antitumor efficacy of reticulol from Streptoverticillium against the lung metastasis model B16F10 melanoma. Lung metastasis inhibition by growth inhibition of melanoma. *Chemotherapy*. 2003;49(3):146-153.
11. Niu S, Liu Q, Xia J-M, et al. Polyketides from the deep-sea-derived fungus *Graphostroma* sp. MCCC 3A00421 showed potent antifood allergic activities. *J Agric Food Chem*. 2018;66(6):1369-1376.
12. Lin J, Yoshida S, Takahashi N. Metabolites Produced by *Streptomyces mobaraensis*. *Agric Biol Chem*. 1971;35(3):363-369.
13. Kiang F-M, Chang S-J, Wu C-L. Naphthalene and isocoumarin derivatives from the liverwort *Wettsteinia inversa*. *Phytochemistry*. 1994;37(5):1459-1461.
14. Indrianingsih AW, Tachibana S. α-Glucosidase inhibitor produced by an endophytic fungus, *Xylariaceae* sp. QGS 01 from *Quercus gilva* Blume. *Food Sci Hum Wellness*. 2017;6(2):88-95.
15. Homans AL, Fuchs A. Direct bioautography on thin-layer chromatograms as a method for detecting fungitoxic substances. *J Chromatogr A*. 1970;51(2):327-329.
16. Alakolanga AGAW, Kumar NS, Jayasinghe L, Fujimoto Y. Antioxidant property and α-glucosidase, α-amylase and lipase inhibiting activities of Flacourtia inermis fruits: characterization of malic acid as an inhibitor of the enzymes. *J Food Sci Technol*. 2015;52(12):8383-8388.
17. Krishnaraju AV, Rao TVN, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. *Int J Appl Sci Eng*. 2005;3(2):125-134.
18. Piyasena KGNP, Wickramarachchi WART, Kumar NS, Jayasinghe L, Fujimoto Y. Two phytotoxic azaphilone derivatives from *Chaetomium globosum*, a fungal endophyte isolated from *Amaranthus viridis* leaves. *Mycology*. 2015;6(3-4):158-160.
19. Nickavar B, Abolhasani L, Izadpanah H. α-Amylase inhibitory activities of six *Salvia* species. *Iran J Pharm Res*. 2008;7(4):297-303.