Isolation of Chromanone and Isobenzofuran Derivatives from a Fungicolous Isolate of *Epicoccum purpurascens*

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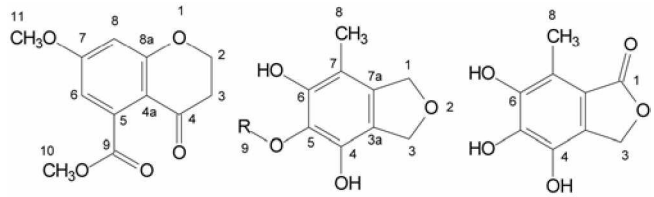
Mycoparasitic and fungicolous fungi are those which attack and colonize other fungal species, and often cause damage to the host fungi. In many cases, at least part of this damage appears to be caused by antifungal metabolites. In spite of such observations, these commonly occurring fungi remain relatively unexplored from a chemical standpoint. As a part of our ongoing studies of mycoparasitic and fungicolous fungi as sources of new antifungal metabolites,[1-3] a fungicolous isolate of *Epicoccum purpurascens* (MYC 1097 = NRRL 37031) was selected for investigation. *Epicoccum purpurascens* Ehrenb. Ex Schlecht (syn. *E. nigrum* Link) has been recorded as a colonist of decaying basidiocarps of larger fungi[4] and has been studied as an antagonist that produces antibiotics[5-8] and/or parasitizes the mycelium of fungal pathogens of crop plants.[9-12] Studies of an organic extract from cultures of *E. purpurascens* MYC 1097 afforded two new metabolites: 7-methoxy-4-oxo-chroman-5-carboxylic acid methyl ester (**1**) and 1,3-dihydro-5-methoxy-7-methylisobenzofuran (**2**). The known compounds 4,5,6-trihydroxy-7-methyl-3*H*-isobenzofuran-1-one (**3**) and 1,3-dihydro-4,6-dihydroxy-7-methylisobenzofuran (**4**) were also obtained. Details of these studies are presented here.

The EtOAc extract of solid-substrate fermentation cultures of *E. purpurascens* was fractionated by Sephadex LH-20 column chromatography. Chromanone metabolite **1** was obtained from the least polar fraction by subsequent silica gel column chromatography. HPLC separation of more polar fractions led to the isolation of the isobenzofuran derivatives **2**-**4**.



1 **2** R = CH_3 **3**
4 R = H

The molecular formula of compound **1** was determined to be $C_{12}H_{12}O_5$ (seven unsaturations) on the basis of HREIMS analysis in combination with ^{1}H and ^{13}C NMR data. Compound **1** showed a simple ^{1}H NMR spectrum containing signals for two methoxy groups, an isolated -OCH_2CH_2- unit, and two *meta*-coupled aromatic protons. The presence of two carbonyl carbons was indicated by ^{13}C NMR signals at δ_C 189.0 and 169.7, and by IR absorptions at ν_{max} 1733 and 1685 cm^{-1}. Analysis of ^{13}C NMR δ-values and HMQC data confirmed the presence of a 1,3-dioxygenated, tetrasubstituted benzene ring. These results suggested that compound **1** has a disubstituted chromanone structure. The locations of the substituents were established primarily on the basis of HMBC and NOE data (Figure 1). An HMBC correlation of H_3-11 to C-7 established the position of the aryl methoxy group, while a correlation of H_3-10 with carboxy carbonyl C-9 indicated the presence of a methyl ester unit. Correlations of the protons of the -OCH_2CH_2-unit with the carbonyl at δ_C 189.0 and two adjacent aryl carbons (C-4a and C-8a) as shown in Figure 1 required formation of a pyranone ring fused to the benzenoid moiety. NOE correlations of H_3-11 with both H-6 and H-8, and of H_3-10 with H-6, supported the regiochemistry shown in **1**, and completed assignment of the structure. Chromanone derivatives have been isolated from a variety of fungi[13] and plants.[14] Among the known naturally occurring chromanones, nearly all have alkyl substituents at the C-2 or C-3 position. 5,7-Dihydroxy-8-methoxy-4-chromanone, previously isolated from a plant source,[15] appears to be the only prior example that is unsubstituted in the pyranone ring. To our knowledge, compound **1** is the first fungal chromanone which bears no substituent at either the C-2 or C-3 position.

Known compound **4**, originally reported as a potent antioxidant metabolite from *Aspergillus terreus*, was isolated as a relatively major constituent of the extract, and identified

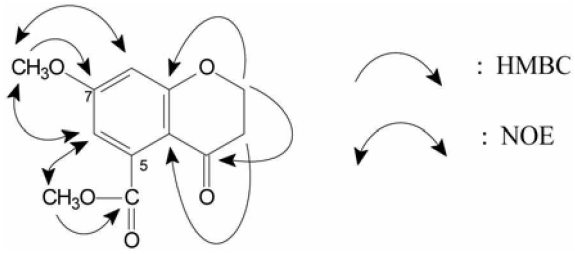


Figure 1. HMBC and NOE correlations for compound **1**.

by comparison of ^{1}H NMR, ^{13}C NMR, EIMS, and melting point data to literature values.[16] Compound **2** showed ^{1}H and ^{13}C NMR data very similar to those of **4**. A 2.2-Hz homobenzylic coupling constant between the two sets of methylene protons (H_2-1 and H_2-3 was also observed, which is consistent with this type of dihydrobenzofuran structure.[17] The only difference was the presence of an additional methoxy group (δ_H 3.73; δ_C 61.0) in place of one of the phenolic OH groups. This was consistent with the molecular formula $C_{10}H_{12}O_4$, which was established by HREIMS. The methoxy group was located at C-5 on the basis of HMBC and 1D NOE data. An HMBC correlation of H_3-9 to the most upfield oxygen-substituted aromatic carbon (C-5), as well as NOE interactions between H_3-9 and both phenolic OH signals, enabled placement of the methoxy group at C-5. The location of the aryl methyl group at C-7 was also supported by HMBC and NOE analysis. Compound **2** has not been previously reported, but the 4,5-dimethoxy analog has been described from another fungal source.[18]

The ^{1}H NMR spectrum of compound **3** ($C_9H_8O_5$ based on HREIMS) contained only two singlets at δ_H 5.11 and 2.41, along with three broad OH peaks (using DMSO-d_6 as the NMR solvent). This observation, together with analysis of ^{13}C NMR data, suggested that **3** is an oxidized version of **4** containing carboxy carbonyl group in place of one methylene unit with, to give an isobenzofuranone structure. The identity of this compound was confirmed as shown in **3** by spectral analysis. This metabolite has been recently encountered as a metabolite of a marine isolate of *Epicoccum* sp., and was assigned the common name epicoccone.[19] Other isobenzofuranones[20] and isobenzofuran acetal[21] metabolites, which have structural similarities to **2-4**, have been previously isolated as metabolites from *Aspergillus* spp.

Despite the source of the isolate and the prior reports of antibiotic activity for metabolites and extracts of *E. purpurascens*, standard disk assays for antifungal effects against *Aspergillus flavus* NRRL 6541 and *Fusarium verticillioides* NRRL 25457 revealed that none of the four compounds isolated showed significant activity at levels up to 200 μg/disk. Biosynthetically, compounds **1-4** are all presumed to be derived from the polyketide pathway, with **1** constructed from five acetate units, and **2-4** arising from four acetate units.

In conclusion, chemical studies of an organic extract of the fungicolous fungus *Epicoccum purpurascens* (MYC-1097 = NRRL 37031) led to isolation of two new metabolites **1**, **2** and two known compounds **3** and **4**. The structures of these compounds were elucidated on the basis of NMR and MS data.

Experimental Section

General experimental procedures. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker DRX-400 instrument. HMBC and HMQC spectra were recorded at 600 MHz (^{1}H dimension, Bruker AMX-600). NMR spectra were referenced to the solvent signals ($CDCl_3$ at δ_H 7.24/δ_C 77.0 and acetone-d_6 at δ_H 2.05/δ_C 29.8). UV measurements were performed with a Varian Cary 100 Bio UV-Vis spectrophotometer. IR measurements employed a FT-IR Perkin Elmer Spectrum BX instrument. Reversed-phase HPLC was performed using a C_{18}-Alltech HS Hyperprep 100 BDS column (250 × 10 mm, 8-μm particles) using a Beckman 110B solvent delivery module and a Beckman 168 diode array detector.

Fungal material. A culture identified as *Epicoccum purpurascens* Ehrenb. ex Schlecht was isolated by D.T.W. as MYC-1097 from an unidentified fungal growth on a dead hardwood branch collected from an area of Live Oak-Palmetto at Alligator Point near Panacea, Florida, by D.T.W. on April 30, 2000. A subculture of this isolate was deposited with the NRRL Collection at the USDA NCAUR and assigned the accession number NRRL 37031. General isolation and fermentation procedures used have been published elsewhere.[17]

Extraction and isolation. The culture was incubated on rice (2 × 50 g) at 25 °C for 30 days and extracted with EtOAc (3 × 500 mL). The resulting EtOAc extract (1.5 g) was partitioned between CH_3CN and hexane to obtain a CH_3CN-soluble mixture (1.1 g) that was fractionated using a Sephadex LH-20 column (110 g) eluting with 1:1 CH_2Cl_2-acetone to provide 20 fractions. Non-polar fraction 2 (4.5 mg) was further purified by passing through a small silica gel pad with 3:2 hexane-EtOAc to remove trace impurities to afford compound **1** (2.2 mg; R_f 0.24 in 3:2 hexane-EtOAc). Reversed-phase HPLC separation of fraction 11 (12 mg) eluting with a 30-100% CH_3CN in H_2O over 30 min yielded compound **2** (2.7 mg). Silica gel column chromatography of fraction 15 (58 mg) using 10:1 $CHCl_3$-MeOH provided compound **4** (37 mg, R_f 0.34 in 9:1 $CHCl_3$-MeOH). Silica gel column chromatography of fraction 16 (25 mg) using 9:1 $CHCl_3$-MeOH provide a subfraction (8.5 mg), which was further purified by reversed-phase HPLC eluting with a 30-100% CH_3CN in H_2O over 30 min to yield compound **3** (4.3 mg; t_R 6.2 min). Known compounds **3** and **4** were identified by analysis of MS and NMR data, and by comparison to literature values.[17,18]

7-Methoxy-4-oxo-chroman-5-carboxylic acid methyl ester (1): pale yellow solid; mp 94-96 °C; UV (MeOH) λ_{max} 225 (ε 11000); IR ($CHCl_3$) ν_{max} 2917, 2846, 1733, 1685, 1603, 1273, 1146 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 6.53 (1H, d, J = 2.4, H-6), 6.44 (1H, d, J = 2.4, H-8), 4.51 (2H, t, J = 6.5, H_2-2), 3.92 (3H, s, H_3-10), 3.82 (3H, s, H_3-11), 2.74 (2H, t, J = 6.5, H_2-3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 189.0 (C-4), 169.7 (C-9), 165.1 (C-7), 163.9 (C-8a), 136.2 (C-5), 112.2 (C-4a), 109.2 (C-6), 102.1 (C-8), 67.2 (C-2), 55.9 (C-10), 52.9 (C-11), 37.4 (C-3); HMBC correlations (H → C#) H_2-2 C-3, C-4, C-8a; H_2-3 → C-2, C-4, C-4a; H-6 → C-4a, C-7, C-8, C-9; H-8 → C-4 ($^4J_{C,H}$), C-4a, C-6, C-7, C-8a; H_3-10 → C-9; H_3-11 → C-7; EIMS (70 eV) m/z 236 (M^+; rel int 40), 208 (32), 205 (27), 178 (23), 165 (16), 150 (100); HREIMS m/z 236.0688 (calcd for $C_{12}H_{12}O_5$, 236.0685).

1,3-Dihydro-4,6-dihydroxy-5-methoxy-7-methylisobenzofuran (2): light brown powder; mp 164-166 °C; UV

(MeOH) λ_{max} 225 (ε 12000), 271 (ε 2700); IR ($CHCl_3$) ν_{max} 3348, 2858, 1376, 1116, 1055 cm^{-1}; ^1H NMR (acetone-d_6, 400 MHz) δ 7.81 (s, C4-OH), 7.54 (s, C6-OH), 4.95 (t, J = 2.2, H_2-3), 4.91 (t, J = 2.2, H_2-1), 3.74 (s, H_3-9), 2.00 (s, H_3-8); ^{13}C NMR (10:1 $CDCl_3$-CD_3OD, 100 MHz) δ 146.7 (C-6), 140.8 (C-4), 134.6 (C-5), 134.1 (C-7a), 116.1 (C-3a), 109.2 (C-7), 73.5 (C-1), 72.1 (C-3), 61.0 (C-9), 11.7 (C-8); HMBC correlations (acetone-d_6, H → C#) H_2-1 C-3a, C-4 ($^3J_{C\text{-}H}$), C-6 ($^3J_{C\text{-}H}$), C-7, C-7a; H_2-3 → C-3a, C-4, C-7 ($^3J_{C\text{-}H}$), C-7a; H_3-8 → C-6, C-7, C-7a; H_3-9 → C-5; C4-OH → C-3a, C-4, C-5; C6-OH → C-5, C-6, C-7; NOE correlations (acetone-d_6, H↔H) H_2-1↔H_3-8; H_3-8↔C6-OH; H_3-9↔C4-OH; H_3-9↔C6-OH; EIMS (70 eV) m/z 196 (M^+; rel int 98), 195 (96), 180 (39), 167 (100), 152 (52); HREIMS m/z 196.0734 (calcd for $C_{10}H_{12}O_4$, 196.0736).

4,5,6-Trihydroxy-7-methyl-3*H*-isobenzofuran-1-one (3): brown solid; mp > 250 °C; UV (MeOH) λ_{max} 222 (ε 25000), 253 (ε 16000); IR ($CHCl_3$) ν_{max} 3164, 1725, 1352, 1284, 1025 cm^{-1}; ^1H NMR (CD_3OD, 300 MHz) δ 5.11 (2H, s, H-3), 2.41 (3H, s, H-8); ^{13}C NMR (3:1 $CDCl_3$-CD_3OD, 100 MHz) δ 172.9 (C-1), 144.2 (C-6), 138.7 (C-4), 136.1 (C-5), 126.5 (C-3a), 117.2 (C-7), 113.5 (C-7a), 66.6 (C-3), 9.1 (C-8); HMBC correlations (H → C#) H_2-3 → C-1 ($^3J_{C\text{-}H}$), C-3a, C-4, C-5 ($^3J_{C\text{-}H}$), C-7a; H_3-8 → C-1 ($^3J_{C\text{-}H}$), C-3a ($^3J_{C\text{-}H}$), C-5, C-6, C-7. Cross peak intensities for correlation of H_3-8 to C-1 and C-3a were weak; HREIMS m/z 196.0373 (calcd for $C_9H_8O_5$ 196.0372); EIMS (70 eV) m/z 196 (M^+; rel int 67), 167 (100).

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