Isolation of an Isocoumarin and an Isobenzofuran Derivatives from a Fungicolous Isolate of *Acremonium crotocinigenum*

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Mycoparasitic and fungicolous fungi are those that parasitize or colonize other fungal species, and often cause damage to the host fungi.[1,2] Our studies of mycoparasitic and fungicolous fungi have led to the isolation of a variety of new bioactive secondary metabolites.[3-8] The occurrence of antifungal metabolites is proving to be common among these fungi, as might be predicted based on their tendency to cause damage to host species. In the course of this project, an isolate of *Acremonium crotocinigenum* (MYC-1590) was subjected to chemical investigation. An organic extract from cultures of this isolate showed potent antifungal activity in disk diffusion assays. Fractionation of the crude EtOAc extract of solid-substrate fermentation culture of MYC-1590 by silica gel column chromatography, followed by repeated reverse phase HPLC, afforded two new metabolites; 6,8-dimethoxy-4,5-dimethyl-3-methyleneisochroman-1-one (**1**) and 5,7-dimethoxy-3,4-dimethyl-3-hydroxy-isobenzofuranone (**2**). Details of the isolation and structure determination of **1** and **2** are presented here.

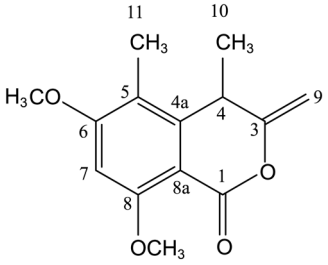
The molecular formula of compound **1** was determined to be $C_{14}H_{16}O_4$ (seven unsaturations) by analysis of HREIMS as well as ^{1}H, ^{13}C, and DEPT NMR data. All 16 protons were bound to carbon based on DEPT results, so no exchangeable OH groups were present. Compound **1** showed simple ^{1}H and ^{13}C-NMR spectra (Table 1) containing signals for an exomethylene group, two aromatic methoxy groups, an isolated aromatic methine, a methyl attached to an sp^3 methine, an ester carbonyl group, and one aromatic methyl group. Accordingly, a bicyclic structure was required for **1** to fulfill the unsaturation requirement. Thus, the ester carbonyl carbon was attributed to a lactone ring.

Analysis of ^{13}C NMR δ values and DEPT data revealed the presence of a 1,3-dioxygenated, pentasubstituted benzene ring. An isolated CH_3-CH-C=CH_2 spin-system corresponding to the C10-C4-C3-C9 unit in **1** was assigned on the basis of ^{1}H-^{1}H COSY data and coupling constants. A long-range correlation between H-9 and one of the exomethylene signals at δ_H 4.63 (H-4, dd, J = 0.4, 1.5 Hz) was observed in the ^{1}H-^{1}H COSY spectrum, indicating that the exomethylene group is linked to the C-9 methine bearing a methyl group at δ_H 1.35 (J = 7.0 Hz). In addition, attachment of the olefinic carbon C-3 to an oxygen atom was evident from the downfield shift (δ_C 157.0) of its ^{13}C NMR resonance.

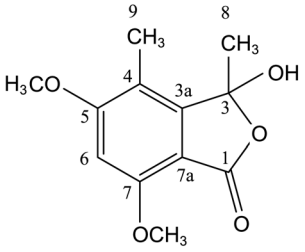
Analysis of HMBC data readily established the regiochemistry of the substituents on the aromatic ring. HMBC correlations of H-7 with C-5, C-6, C-8, and C-8a and of the aryl methyl signal with C-5, C-6, and C-4a indicated that the two methoxy groups and the aryl methyl group were positioned at C-6, C-8, and C-5, respectively, a situation analogous to that of several previously reported isocoumarins[9-11] and consistent with a presumed polyketide biogenetic origins. Thus, compound **1** was identified as 6,8-dimethoxy-4,5-dimethyl-3-methyleneisochroman-1-one. Compound **1** is most closely related to halorosellin A,[12] differing in the replacement of a glucose moiety at C-8 with a methoxy group, and addition of a second methoxy unit at C-6 in place of a free OH group.

Table 1. ^{1}H (400 MHz) and ^{13}C (100 MHz) NMR spectral data of 6,8-dimethoxy-4,5-dimethyl-3-methyleneisochroman-1-one (**1**) and 5,7-dimethoxy-3,4-dimethyl-3-hydroxy-isobenzofuranone (**2**)

position	**1**		**2**	
	δ_H (mult., J in Hz)	δ_C	δ_H (mult., J in Hz)	δ_C
1		160.0		165.0
3		157.0		104.5
3a				150.4
4	3.75 (q, 7.0)	35.8		114.1
4a		144.6		
5		114.0		166.4
6		163.0	6.38 (s)	95.8
7	6.40 (s)	93.7		160.0
7a				105.6
8		161.7	1.82 (s)	25.7
8a		104.0		
9	4.41 (d, 1.5)	93.4	2.21 (s)	10.3
	4.63 (dd, 0.4, 1.5)			
10	1.35 (d, 7.0)	21.0		
11	2.10 (s)	10.0		
5-OCH_3			3.90 (s)	56.3
6-OCH_3	3.89 (s)	55.8		
7-OCH_3			3.92 (s)	56.4
8-OCH_3	3.94 (s)	56.2		



1



2

EIMS, ^{13}C NMR, and DEPT data for compound **2** indicated the molecular formula $C_{12}H_{14}O_5$ (six unsaturations) and the presence of one exchangeable OH group. The ^{1}H and ^{13}C NMR spectra of **2** were very similar to those of **1**. However, the ^{1}H and ^{13}C NMR signals for the exomethylene unit were missing in **2**. Furthermore, a dioxygenated quaternary carbon signal at δ_C 104.5 and a methyl singlet at δ_H 1.82 appeared in the ^{1}H NMR spectrum of **2** in place of the CH-CH_3 signals in the spectrum of **1**. The appearance of the carbonyl at δ_C 165.0 in **2**, when combined with molecular formula, suggested the presence of a γ-lactone ring instead of the δ-lactone ring found in **1**. On the basis of these data, the structure of **2** was assigned as 5,7-dimethoxy-3,4-dimethyl-3-hydroxy-isobenzofuranone (**2**).

Some isobenzofuranone derivatives such as 4,5,6-trihydroxy-7-methyl-3*H*-isobenzofuran-1-one,[13] epicoccone,[14] 3-acetyl-7-hydroxy-5-methoxy-3,4-dimethyl-3*H*-isobenzofuran-1-one,[15] and isopestacin[16] have been reported from fungi. The compound most closely resembling **2** is 3-acetyl-7-hydroxy-5-methoxy-3,4-dimethyl-3*H*-isobenzofuran-1-one, which was isolated from the marine fungus *Halorosellinia oceanica*,[15] and contains an acetate group at C-8 and a hydroxyl group at C-7. The ^{13}C NMR data of the aromatic moiety in **2** was very similar to that of 3-acetyl-7-hydroxy-5-methoxy-3,4-dimethyl-3*H*-isobenzofuran-1-one, which supported the assignment of structure **2**. To the best of our knowledge, compound **2** is the only example of a naturally occurring isobenzofuranone having oxygenation at C-3.

Compounds **1** and **2** showed no activity in standard agar disk diffusion assays at 100 μg/disk against *Bacillus subtilis* (ATCC 6051), *Staphylococcus aureus* (ATCC 29213), *Escherichia coli* (ATCC 25922), and *Candida albicans* (ATCC 14053). Compound **1** was also inactive against *Aspergillus flavus* (NRRL 6541) at 200 μg/disk, but did show activity against *Fusarium verticillioides* (NRRL 25457), affording a 30-mm zone of inhibition at 100 μg/disk.

Compound **1**, like most natural isocoumarins, is proposed to be biogenetically derived from acetate via the polyketide pathway.[17] A straightforward pentaketide biosynthetic pathway for the formation of halorosellin A aglycone has been postulated in the literature[12] and is presumably the same pathway involved in the formation of **1**. While compound **2** is biogenetically related to **1**, it seems more likely to arise from decarboxylation of a herbaric acid-type precursor than directly from a halorosellin precursor.

In summary, chemical studies of an organic extract of the fungicolous fungus *Acremonium crotocinigenum* (MYC-1590) led to the isolation of new metabolites **1** and **2**. The structures of both compounds were elucidated on the basis of NMR and MS data.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in $CDCl_3$ and chemical shifts were referenced relative to the corresponding signals (δ_H 7.24/δ_C 77.23). ^{1}H NMR data were recorded at 400 MHz (Bruker DRX-400) or 600 MHz (Bruker AMX- 600). ^{13}C NMR data were recorded at 100 MHz (Bruker DRX-400). All 2D NMR data were recorded at 600 MHz (^{1}H dimension). EIMS and HREIMS data were recorded using a Fisons Autospec double focusing mass spectrometer. All the HPLC separations were carried out using a Alltech HS Hyperprep 100 BDS C18 column, (10 × 250 mm) at a flow rate of 2 mL/min.

Fungal Material. The culture employed in this work (MYC 1590) was originally isolated by D.T.W. from a basidioma of *Earliella scabrosa* found on a dead hardwood branch, alien wet forest, Hilo Zoo, Hawaii Co., HI. The isolate was identified as *Acremonium crotocinigemun* (Schol-Schwarz) W. Gams, and a subculture was deposited in the NRRL Collection at the USDA NCAUR in Peoria, IL, USA under the accession number NRRL 40192. General fermentation and extraction procedures employed have been described elsewhere.[4]

Isolation. The crude ethyl acetate extract (1.5 g) obtained from eight 500-mL fermentation flasks each containing 50 g of rice was first partitioned between hexanes (3 × 100 mL) and CH_3CN (50 mL), and the CH_3CN-soluble portion (0.231 g) was fractioned by silica gel column chromatography using a hexanes-EtOAc solvent gradient. The last fraction was subjected to Sephadex LH-20 column chromatography to afford five subfractions. The subfraction eluted with 70:30 dichloromethane-methanol (70 mg) was further separated by semipreparative reversed-phase HPLC (20 to 100% CH_3CN in H_2O over 45 min) to afford compound **1** (17 mg). Another subfraction eluted with 50:50 acetone-methanol was further subjected to semipreparative reversed-phase HPLC (40 to 70% CH_3CN in H_2O over 30 min) to provide compound **2** (8 mg).

6,8-Dimethoxy-4,5-dimethyl-3-methyleneisochroman-1-one (1): Colorless needles; ^{1}H NMR and ^{13}C NMR, see Table 1; ^{1}H-^{1}H COSY correlations ($CDCl_3$, H-# → H-#) H-4 ↔ H-10 and H-9a; H-9a ↔ H-9b and H-4; HMBC data: H-4 → C-3, 4a, 5, 8a, 9, 10; H-7 → C-5, 6, 8, 8a; H_2-9 → C-3, 4; H_3-10 → C-3, 4, 4a; H_3-11 → C-4a, 5, 6; H_3-12 → C-6; H_3-13 → C-8; HREIMS *m/z* 248.1046 $[M]^+$, calcd for $C_{14}H_{16}O_4$, 248.1049.

5,7-Dimethoxy-3,4-dimethyl-3-hydroxy-isobenzofuranone (2): Colorless needle; ^{1}H NMR and ^{13}C NMR, see Table 1; EIMS *m/z* 238 $[M]^+$.

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