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Structural Elucidation of a Putative Conidial Pigment Intermediate in *Aspergillus Parasiticus*

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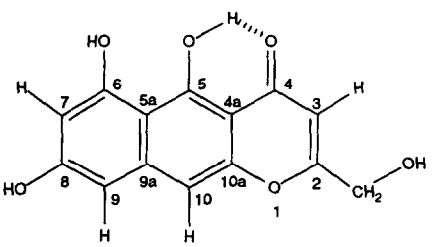
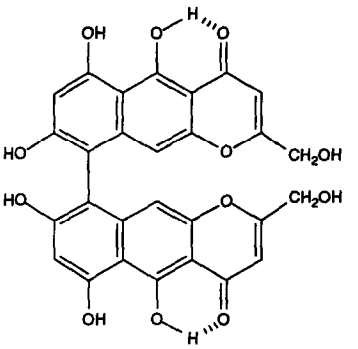
Abstract: A novel, hydroxylated naphtho[2,3-b]pyran has been isolated from a laccase-deficient strain of *Aspergillus parasiticus* and characterized through spectroscopic means.



1, Parasperone A



2, Ustilaginoidin C

Asexual reproduction in several species of the ascomycetous fungus *Aspergillus* (e.g. *parasiticus*, *flavus*, and *nidulans*) culminates in the production of green pigmented spores. These spores are more resistant to UV light and other environmental stresses than hyaline (pigment-free) spores.[1] The native green pigments are polar high molecular weight materials that are refractory to direct structural analysis. However, orange pigments that accumulate in strains deficient in the *p*-diphenol oxidase, laccase (E.C. 1.10.3.2), are believed to be intermediates in the native pigment's biosynthesis.[2]

We have achieved the first isolation and characterization of the novel hydroxymethyl naphthopyranone, parasperone A (**1**), produced from a mutant strain of *A. parasiticus*. Parasperone A (**1**) is the monomer of ustilaginoidin C (**2**), a compound with demonstrated antitumor activity[3a] found in extracts of *Claviceps virens*.[3b] Although these two materials are produced by different organisms, formation of the hydroxymethyl functionality apparently precedes oxidative dimerization, suggesting

that **1** may be a precursor in the biosynthesis of **2**.

Spores produced by a laccase deficient strain of *A. parasiticus* (DB291*ol*), derived from ATCC 74022, were extracted with ether using a Normag liquid-liquid extraction apparatus. The ethereal solution was concentrated at reduced pressure and the crude extract was purified by preparative HPLC using a C-18 reverse-phase column (Nest Group, 2 cm x 25 cm, eluted with a gradient of water/acetone, 60% to 5%) to give the primary orange pigment, parasperone A, **1**. Two other pigments, parasperone B and C, were also isolated and although they have yet to be fully characterized they appear to be regioisomeric dimers of **1**.[4]

Structure elucidation of **1** was particularly challenging as the isolation procedure afforded less than 5 mg of material. Derivatization of **1** with N,O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) gave a bis(TMS) derivative with a molecular ion (M^+, FD-MS) at 418.1292 *m/e* (calculated for $C_{14}H_8O_6$ + 2(TMS): 418.1263) which suggested a molecular formula of $C_{14}H_{10}O_6$. The UV/visible and IR absorptions shown in **Table 1** are characteristic of the linear 4H-naphtho[2,3-b]pyran-4-one ring system and are distinguished from the angular 4H-naphtho[1,2-b]pyran-4-ones such as flavasperone **3**.[5] The ^{13}C and ^1H NMR spectral assignments for **1** are listed in **Table 2**. Deuterium exchange caused the disappearance of the ^1H resonances at 15.7, 10.2, 9.8, and 5.8 ppm, which indicated the presence of four

Table 1. UV and IR data for **1**

UV $\lambda_{max}^{CH_3CN}$ (nm)	IR (cm^{-1})
408	3400-3200
325	1658, 1620
277	1588, 1477
250	1402, 1372
223	1342, 1292
	1265, 1160
	1130

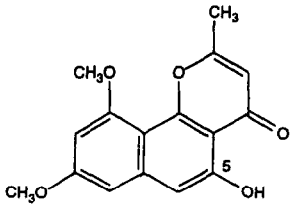
Table 2. Carbon and Proton Assignments for **1**[a]

Position	^{13}C δ ppm	^1H δ ppm (mult., J (Hz))	Position	^{13}C δ ppm	^1H δ ppm (mult., J (Hz))
2	172.5	—	9	100.8	6.3 (d,2.0)
3	103.4	6.2 (s)	9a	140.5	—
4	183.5	—	10	100.0	7.0 (s)
4a	102.3	—	10a	151.8	—
5	162.5	—	CH_2OH	59.9	4.4 (d,5.9)
5a	105.6	—	$CH_2O\underline{H}$	—	5.8 (t,5.9)
6	158.6	—	5-OH	—	15.7 (bs)
7	100.8	6.6 (d,2.0)	6-OH	—	9.8 (s)
8	160.9	—	8-OH	—	10.2 (s)

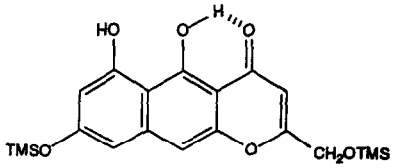
a) Proton (500.01 MHz) and carbon (125.75 MHz) NMR spectra were obtained in DMSO-d_6 and are referenced to residual DMSO-d_5.

hydroxyl groups. Likewise, the resonances at 10.2 and 5.8 were absent in the bis(TMS) derivative, which suggested structure **4** for this material. The unusually high resonance at 15.7 ppm also suggested the linear structure since the chemical shift for the hydrogen-bonded 5-OH in **3** is at 12.9 ppm.

The structure of **1** was further elucidated through the use of 2D NMR spectroscopic techniques. An Attached Proton Test (APT) indicated one methylene, four tertiary and eight quaternary carbons. Heteronuclear Multiple Quantum Coherence (HMQC) spectroscopy[6] provided one-bond proton-carbon connectivities and thus assignment of aromatic carbons C-3, C-7, C-9, and C-10. Note that only 13



3, Flavasperone



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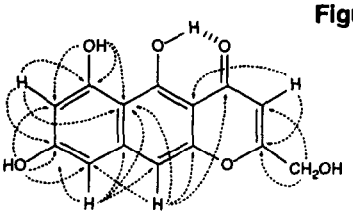
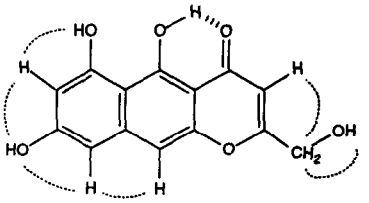
resonances were observed in the carbon spectrum of **1**, whereas the molecular formula suggests 14 carbons. The HMQC experiment established that the carbon resonance at 100.8 ppm is in fact a coincidental overlap for carbons C-7 and C-9. Long-range proton-carbon connectivity correlations and spectral assignments for all quaternary carbons, except C-5, were established through Heteronuclear Multiple Bond Coherence (HMBC) spectroscopy.[6] **Figure 1** shows the qualitative multiple-bond interactions found by this technique. Correlations for the C-5 phenolic carbon were obscured by spectral noise and not observed. Interactions noted in the NOESY spectrum are also shown in **Figure 1**.

Figure 1



HMBC Correlations

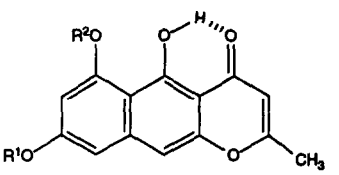


NOE interactions

The proton and carbon spectra for **1** and **2** are nearly identical. Likewise, the structure of **1** bears a remarkable similarity to the fully characterized monomeric naphthopyranones, rubrofusarin (**5**)[7a] and rubrofusarin B (**6**),[7b] which are the structural units in the aurosperones,[8a] nigerones,[8b] and many other regioisomeric dimers.[9]

Based on the structural similarity to the heptaketide derived rubrofusarin (**5**),[7a] we propose that parasperone A is also derived from a heptaketide.[10] Preliminary results indicate that similar orange pigments are also found in the spores of other *Aspergilli* (e.g., *nidulans*).

A number of other related naphthopyrone dimers have been reported to exhibit antitumor activities.[1b] The characterization of additional, unique members of this family will allow a continued examination of structure-activity relationships.[1]



5, Rubrofusarin ($R^1 = CH_3$, $R^2 = H$)

6, Rubrofusarin B ($R^1 = R^2 = CH_3$)

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