10-Methoxydihydrofuscin, Fuscinarin, and Fuscin, Novel Antagonists of the Human CCR5 Receptor from *Oidiodendron griseum*

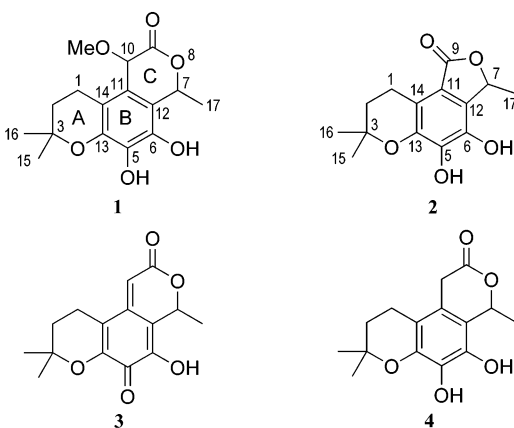K. Yoganathan,* Christine Rossant,[†] Siewbee Ng, Yicun Huang, Mark S. Butler, and Antony D. Buss

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Two new compounds, 10-methoxydihydrofuscin (**1**) and fuscinarin (**2**), and one known compound, fuscin (**3**), have been isolated from the soil fungus *Oidiodendron griseum*. These compounds were found to compete effectively with macrophage inflammatory protein (MIP)-1α for binding to human CCR5, an important anti HIV-1 target that interferes with HIV entry into cells. The structures of these compounds were elucidated by spectroscopic methods.

Chemokines are small proteins (7–16 kDa) that act through G protein-coupled receptors to regulate a variety of physiological and pathophysiological processes.[1] The human immunodeficiency virus Type 1 (HIV-1) uses chemokine receptors (principally CCR5 and CXCR4) as co-receptors with CD4 to gain entry into target cells. The binding of the gp120 subunit of the viral envelope glyco-protein to CD4 causes a conformational change in the viral envelope glycoprotein that results in the exposure of the domain on gp120 for binding to CCR5 or CXCR4. As a consequence, the gp41 subunit inserts into the target cell membrane and the fusion of the virus with the target cell is initiated.[2] Therefore, a molecule that binds to the CCR5 receptor could potentially prevent HIV-1 entry into cells, making CCR5 an attractive drug target.[3]

An extract from the fungus *Oidiodendron griseum* (a mitosporic fungus) showed activity against CCR5 in a scintillation proximity assay (SPA). Bioassay-guided frac-tionation led to the isolation of two new compounds, **1** and **2**, and the known compound fuscin (**3**). Fuscin (**3**), which has been previously isolated from *O. fuscum*,[4] O. *rhodoge-num*,[5] and *Potebniamyces gallicola*,[6] was characterized by comparing its ^{1}H and MS spectral data with those in the literature.[5,6]

Table 1. [1]H (500 MHz) and ^{13}C (125 MHz) NMR Data for 10-Methoxydihydrofuscin (**1**), Fuscinarin (**2**), and Dihydrofuscin (**4**)[6] in CD₃OD

position	**1** [13]C[a]	**1** [1]H[a] (mult., *J* in Hz)	**2** [13]C[a]	**2** [1]H[a] (mult., *J* in Hz)	**4**[6] [1]H[b] (mult., *J* in Hz)
1a	19.2	2.67 (ddd, 16.5, 7, 7)	19.1	3.02 (t, 7)	2.55 (t, 6)
1b		2.78 (ddd, 16.5, 7, 7)			
2	33.6	1.84 (t, 7)	32.9	1.81 (t, 7)	1.83 (t, 6)
3	75.7		76.4		
5	135.6		138.6		
6	139.9		140.9		
7	76.9	5.71 (q, 7)	76.4	5.45 (q, 7)	5.84 (q, 7)
9	170.8		173.4		
10	74.1	4.68 (s)			3.38 (d, 20)
					3.68 (d, 20)
11	119.9		113.4		
12	118.6		132.9		
13	143.6		144.4		
14	113.2		113.8		
15	26.5	1.33 (s)	26.5	1.36 (s)	1.31 (s)
16	26.9	1.36 (s)	26.5	1.36 (s)	1.35 (s)
17	24.1	1.63 (d, 7)	19.6	1.58 (d, 7)	1.57 (d, 7)
10-OMe	58.4	3.52 (s)			

[a] Assignments based on COSY, multiplicity-edited HSQC, and HMBC. [b] CD₃OD, 100 MHz; assignments based on ^{1}H NMR.

C=C (1624 cm⁻¹). The ^{13}C NMR spectrum of **1** (Table 1) displayed 16 carbon signals: one carbonyl (δ_C 170.8), seven other quaternary carbons, two oxymethines, two aliphatic methylenes, three methyls, and one methoxy group were identified using a multiplicity-edited HSQC[7] NMR experi-ment. The ^{1}H NMR spectrum of **1** showed signals for two oxymethine groups (δ_H 5.71, q; 4.68, s), two isolated methylene groups (δ_H 2.67, ddd and 2.78, ddd; 1.84, t, 2H), one methoxy group (δ_H 3.52, s), and two tertiary and one secondary methyl groups (δ_H 1.33, s; 1.36, s; and 1.63, d). These groups were somewhat similar to that previously reported for dihydrofuscin (**4**) except for the two signals at δ_H 3.52 and 4.68 in place of the isolated methylene protons at δ_H 3.38 and 3.68 in **4**.[6] Comparison of the molecular formula of **1** with that of **4** suggested that **1** was the C-10 methoxy derivative of dihydrofuscin (**4**). This was confirmed by the observation of ³*J* HMBC correlations from 10-OMe to C-10 and from H-10 to C-9. The ^{13}C NMR resonance of C-10 at δ_C 76.9 also supported this assignment, as it was α to both an oxygen and a carbonyl function. The relative configuration between chiral centers at C-7 and C-10 was investigated by 1D ROSEY NMR experiments. Selective

Compound **1** was obtained as an oil, and its molecular formula established using negative HR-ESIMS. The IR spectrum showed characteristic absorption bands for OH (broad, 3401 cm⁻¹), lactone (1731 cm⁻¹), and aromatic ring

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refocusing of H-7 gave an enhancement to 10-OMe, while selective refocusing of H-10 gave enhancements to H-1a, H-1b, and 10-OMe. The lack of a correlation between H-7 and H-10 was not surprising given their 1,4-arrangement. Selective refocusing of 10-OMe gave a weak enhancement to H_3-17 and vice versa, which allowed a *cis* assignment between these two groups to be *tentatively* proposed.

Compound **2** had a molecular formula $C_{14}H_{16}O_5$ as indicated by its mass spectrum. The ^1H and ^{13}C NMR spectral data (Table 1) showed that **2** resembled 10-methoxydihydrofuscin (**1**) in rings A and B, but substantial changes have occurred in ring C. Although the molecular formula of **2** differed from **1** ($C_{16}H_{20}O_6$) by the absence of the methoxy and methine groups, **2** had the same degree of unsaturation as **1**. Since the NMR spectra ruled out a double bond in ring C, a reduction in ring size was implied and linkage of C-7 and C-11 via a lactone was feasible. This was supported by HMBC correlations from H-7 to C-9, C-11, and C-12, and the structure was assigned as shown. Fuscinarin (**2**) represents the first example of a new class of isobenzofuranones.

10-Methoxydihydrofuscin (**1**), fuscinarin (**2**), and fuscin (**3**) effectively competed with MIP-1α for binding to human CCR5 and showed IC_{50} values of 154, 80, and 21 μM, respectively, in the SPA binding assay. The increased activity of fusin (**3**) compared to **1** and **2** may be due to the presence of the isochromene-5,9-dione unit in **3**, which has multiple reactive sites. These compounds were not considered for further biological evaluation.

Experimental Section

General Experimental Procedures. General experimental procedures have been reported elsewhere.[8]

Microorganism and Fermentation. The fungal strain *O. griseum* is a soil fungus and is deposited in the MerLion Pharmaceuticals culture collection (F31027). The strain was subcultured on malt extract agar (CM057B, Oxoid) for 7 days at 24 °C. It was used to inoculate 250 mL Erlenmeyer flasks each containing 50 mL of seed medium composed of 0.4% glucose, 1% malt extract, and 0.4% yeast extract. The pH of the medium was adjusted to 5.5 before sterilization. The seed culture was incubated for 5 days at 24 °C on a rotary shaker at 200 rpm. A volume of 5 mL of seed culture was used to inoculate 50 mL of liquid medium in a 250 mL flask. The liquid medium is composed of 0.4% yeast extract, 2% glucose, and 2% oatmeal. The pH was adjusted to 7.5 and autoclaved at 121 °C for 30 min. The fermentation was carried out for 9 days at 24 °C at 200 rpm.

Biological Assays. CCR5 receptor binding activity was determined in a 96-well SPA assay[9] format using [^{125}I]-human MIP-1α (Amersham Biosciences) and membranes prepared from Chinese hamster ovary (CHO) cells overexpressing the human CCR5 receptor. The samples were dissolved in 12.5% aqueous DMSO and incubated with 12 μg membranes, 0.17 nM [^{125}I]-MIP-1α, and 0.25 mg of wheat germ agglutinin-SPA beads (Amersham Biosciences) in assay buffer (50 mM Hepes, 1 mM $CaCl_2$, 1 mM $MgCl_2$, 1% BSA, and a protease inhibitor cocktail) for 5 h at room temperature with shaking. Radioactivity (total binding) was measured after a 2 h bead settling period. Nonspecific binding was defined in the presence of 1 μM recombinant human MIP-1α (Peprotech).

Extraction and Isolation. The freeze-dried fermentation broth (2 L) was extracted three times with CH_2Cl_2−MeOH (1:1) and evaporated to dryness under vacuum. The dry extract (3 g) was partitioned three times between hexane and 90% MeOH in H_2O (1:1). The 90% MeOH portion was adjusted to 70% with H_2O and partitioned with CH_2Cl_2 (3×). The active MeOH fraction (1.5 g) was subjected to reversed-phase preparative HPLC using isocratic elution (30% CH_3CN in 0.1% HCOOH, 10 mL/min, 58 min) to give compounds **1** (7 mg), **2** (12 mg), and fuscin (**3**) (1.5 mg). Fuscin (**3**) was characterized by comparing the ^1H and MS spectral data with those in the literature.[5,6]

10-Methoxydihydrofuscin (1): oil; $[\alpha]^{25}_D$ −4.4° (*c* 0.66, MeOH); UV (MeOH) λ_{max} (log ϵ) 215 (4.10), 349 (3.26) nm; IR ν_{max} (NaCl, film) 3401, 2977, 1731, 1624 cm^{-1}; ^1H NMR (CD$_3$OD, 500 MHz, Table 1); ^{13}C NMR (CD$_3$OD, 125 MHz, Table 1); ESIMS (negative) m/z 307 [M − H]$^-$; HRESIMS (negative) m/z 307.1158 (calcd for $C_{16}H_{19}O_6$, 307.1176, [M − H]$^-$).

Fuscinarin (2): oil; $[\alpha]^{25}_D$ −54° (*c* 1.14, MeOH); UV (MeOH) λ_{max} (log ϵ) 220 (4.17), 278 (3.66) nm; IR ν_{max} (NaCl, film) 3400, 2978, 1730, 1628 cm^{-1}; ^1H NMR (CD$_3$OD, 500 MHz, Table 1); ^{13}C NMR (CD$_3$OD, 125 MHz, Table 1); ESIMS (negative) m/z 263 [M − H]$^-$; HRESIMS (negative) m/z 263.0930 (calcd for $C_{14}H_{15}O_5$, 263.0914, [M − H]$^-$).

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