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Synthesis and single crystal X-ray analysis of two griseofulvin metabolites

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ABSTRACT

The two phenols, 6-*O*-desmethyl griseofulvin and 4-*O*-desmethyl griseofulvin are metabolites of the antifungal drug griseofulvin. Herein, we present an improved synthesis of the 6-phenol derivative, and an unequivocal proof of both structures by single-crystal X-ray analysis.

The natural product griseofulvin (**1**) (see Fig. 1) was first isolated by Oxford et al. in 1939[1] and later shown to possess antifungal properties.[2] This antifungal agent is still in clinical use today[3] and is the only orally administered drug approved by the Food and Drug Administration for the treatment of tinea capitis (ringworm of the scalp).[4] Recently, griseofulvin has received renewed attention due to reports of both antiproliferative effects in cancer cells[5–7] as well as suppression of hepatitis C replication.[8] As a result of its notoriously low water solubility, griseofulvin is furthermore, often used as a benchmark compound in formulation studies and in the development of drug delivery systems.[9]

The metabolism of griseofulvin has been studied both in vitro[10] and in vivo and reported in several publications. In addition to studies in fungi,[11] the in vivo metabolism of griseofulvin has been investigated in rats,[12] mice,[13] rabbits,[14] dogs,[15] and man.[16,17] Known important metabolites of griseofulvin include 6-*O*-desmethylgriseofulvin (**2**) and 4-*O*-desmethylgriseofulvin (**3**), but their structures have never been proven unambiguously. In the literature, it is commonly merely stated that the metabolites were compared with authentic samples.[11–13,15,17] Others[10,14] have used spectroscopic properties and melting points to identify the structures by comparing these data with earlier work.[18–21] We present herein, the synthesis and crystal structures of both 6-*O*-desmethylgriseofulvin (**2**) and 4-*O*-desmethylgriseofulvin (**3**), which provide final verification of the structural assignments.

6-*O*-Desmethylgriseofulvin (**2**) was first synthesized by Arkley et al. in six steps with an overall yield of 14% (Scheme 1).[22] To confirm the outcome of these transformations, the synthetic route was reproduced and we were actually able to isolate a small amount of **2** at step three (Scheme 1). The lengthy synthesis and poor yield of this route prompted us to search for a more convenient method to access **2**. Thus, we were pleased to obtain the desired phenol in 29% yield after the treatment of griseofulvin (**1**) with LiI in pyridine at 115 °C (Scheme 2).[22] The synthesis of 4-*O*-desmethylgriseofulvin

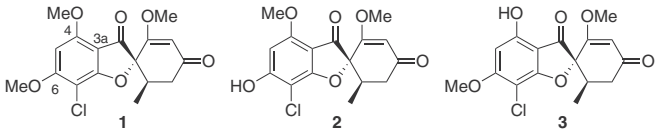
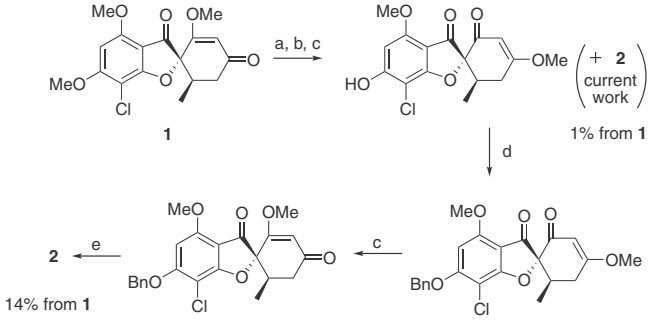


Figure 1. The structures of griseofulvin (**1**), 6-*O*-desmethylgriseofulvin (**2**) and 4-*O*-desmethylgriseofulvin (**3**).



Scheme 1. Reagents: (a) HOAc, 2 M H_2SO_4; (b) 0.5 M NaOH; (c) 2,2-dimethoxypropane, *p*-toluenesulfonic acid, MeOH; (d) K_2CO_3, BnBr, acetone; (e) 5% Pd/C, H_2, EtOAc.

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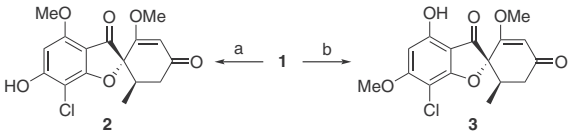

Scheme 2. Reagents: (a) LiI, pyridine, 115 °C, (29%); (b) MgI_2, Et_2O, toluene, (98%).

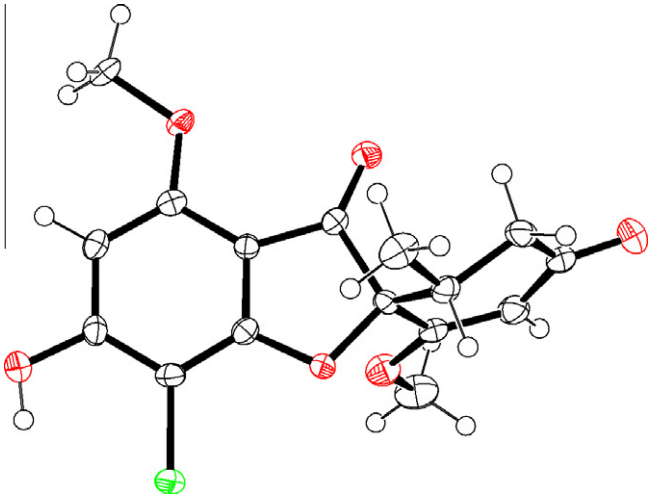

Figure 2. ORTEP view of 6-*O*-desmethylgriseofulvin (**2**).

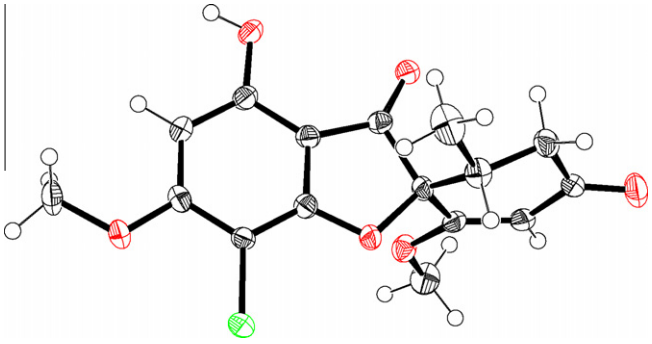

Figure 3. ORTEP view of 4-*O*-desmethylgriseofulvin (**3**).

(**3**) was performed by treatment of **1** with MgI_2 in a mixture of diethyl ether and toluene (Scheme 2), a slight modification of the procedure originally published by Arkley et al.[23]

The structures of **2** and **3** were confirmed unequivocally by the use of single-crystal X-ray analysis (Figs. 2 and 3).[24] It is not possible to distinguish between the 4 and 6 methoxy groups of **1** by gHMBC as no ^4J correlation is observed. The ^{1}H NMR spectrum of **2** (see Supplementary data) does not exhibit a signal for the phenolic hydroxy group, due to rapid proton exchange, and thus no heteronuclear correlations can be used to aid in the assignment of the spectrum. For **3**, the phenolic proton is observed (see Supplementary data) and the gHMBC contains a single $^3J_{HC}$ correlation to C-3a, confirming the position of the phenol. The UV and fluorescence spectra of **2** and **3** were all but identical, and despite small differences in the MS–MS spectra (see Supplementary data), the retention time[25] is still the most reliable and sensitive analytical method for distinguishing the two phenols.

Acknowledgment

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Supplementary data

Supplementary data (experimental procedures, characterization, and purity data, HPLC traces, NMR, UV, and MS–MS spectra for compounds **2** and **3**, and crystallographic information in cif format) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.08.095

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23. *Synthesis of* **2**. Griseofulvin (10 mg, 0.03 mmol) and LiI (4.7 mg, 0.04) were dissolved in pyridine (0.5 mL) and heated to 115 °C. After 16 h, the reaction was allowed to reach 20 °C and satd aq NH_4Cl (2 mL) was added. The mixture was extracted with EtOAc (3 × 3 mL), and the combined organic phases were dried ($MgSO_4$) and concentrated. Purification was performed on a Luna HPLC column (250 × 10 mm, 5 μm, C-18) using 5 mL/min H_2O/CH_3CN (isocratic run at 65:35, for 15 min) as the mobile phase to yield **2** (2.8 mg, 29%) as a yellow oil, which was crystallized from EtOAc and heptane.
24. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication nos. CCDC 775177 (**2**) and CCDC 775176 (**3**). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223 336033 or email: deposit@ccdc.cam.ac.uk).
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