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### Toxigenic Molds in Water-Damaged Buildings: Dechlorogriseofulvins from *Memnoniella echinata*

Bruce B. Jarvis,\* Yihong Zhou, Jian Jiang, and Shengjun Wang

Department of Chemistry and Biochemistry, University of Maryland, College Park, Maryland 20742

W. G. Sorenson

Division of Respiratory Disease Studies, NIOSH, Morgantown, West Virginia 26505

E.-L. Hintikka

National Veterinary and Food Research Institute, Helsinki, Finland

M. Nikulin

Faculty of Veterinary Medicine, Veterinary Microbiology and Epidemiology, University of Helsinki, Finland 00014

P. Parikka

Agricultural Research Center of Finland, Institute of Plant Protection, Jokioinen, Finland 31600

Ruth A. Etzel

National Center for Environmental Health, Centers for Disease Control and Prevention, Department of Health and Human Services, Atlanta, Georgia 30341

Dorr G. Dearborn

Department of Pediatrics, Rainbow Babies and Childrens Hospital, Case Western Reserve School of Medicine, Cleveland, Ohio 44106

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**Abstract:** An investigation of a cluster of cases of pulmonary hemosiderosis in infants in Cleveland, OH, led to the isolation of many isolates of *Stachybotrys atra* and two isolates of a related toxigenic fungus, *Memnoniella echinata*. *M. echinata* produces two cytotoxic trichothecene mycotoxins, trichodermol (**1a**) and trichodermin (**1b**), as well as several griseofulvins. Dechlorogriseofulvin (**2a**) and epidechlorogriseofulvin (**2b**) were the major compounds isolated. This is the first report of a fungus outside the *Penicillium* genus producing griseofulvins.

There has been increasing concern in recent years about the health effects associated with the growth of molds in water-damaged buildings.<sup>1,2</sup> Although the main problems associated with biologics in damp dwellings appear to be allergenic in nature, there are instances of human intoxications caused by fungal toxins (mycotoxins) produced by molds growing in wet buildings.<sup>3,4</sup> A study of an unusual cluster of cases of acute pulmonary hemosiderosis in infants, which resulted in several deaths, in Cleveland, OH, pointed to home dampness as a common factor. An investigation showed that all these homes had varying levels of contamination by *Stachybotrys atra*,<sup>5</sup> a fungus long associated with the deaths of livestock that ingested *S. atra*-contaminated feed (Etzel, R. A.; Montana, E.; Sorenson, W. G.; Kullman, G. J.; Allan, T.; Miller, J. D.; Jarvis, B. B.; Dearborn, D. G. Unpublished results). Preliminary data strongly support the contention that exposure to spores of *S. atra* is the principal cause of the pulmonary hemosiderosis observed in these infants.<sup>5</sup>

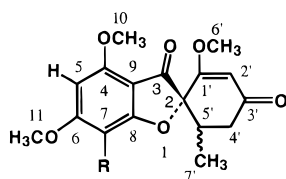
*Memnoniella echinata* (Riv.) Galloway is a fungus closely related to *S. atra* and is sometimes found growing along with *S. atra*.<sup>6</sup> In the course of isolating *S. atra* from the homes in Cleveland,<sup>7</sup> we obtained two isolates of *M. echinata* whose cultures proved cytotoxic against fetal feline lung cells.<sup>8</sup> One isolate (to be deposited with the ATCC under the designation JS638) was grown on rice for 30 days, extracted with methanol, and chromatographed to give a number of fractions.

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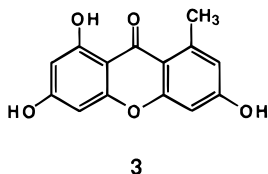
Several chromatographic fractions were highly cytotoxic; two of these fractions yielded trichodermol (**1a**) and trichodermin (**1b**). One major fraction of low cytotoxicity yielded two compounds that were separated by preparative reversed-phase HPLC to give dechlorogriseofulvin (**2a**)<sup>9</sup> and epidechlorogriseofulvin (**2b**), whose structure was established unequivocally by X-ray crystallography.<sup>10,11</sup> Compounds **2a** and **2b** were isolated in substantial quantity (0.5 g each from 12 g of crude extract). The X-ray analysis through enantiomorph-polarity estimation<sup>12</sup> established that **2b** is C-2*S*,C-5'*S*. Although griseofulvins **2a** and **2c** have been isolated from several species of *Penicillium*,<sup>13</sup> the epigriseofulvins (i.e., the C-5'*S* congeners) have not been reported as natural products. Epigriseofulvin (**2**: R = Cl; C-5'*S*) has been synthesized in racemic form.<sup>14</sup> In addition to **2a** and **2b**, we also found minor amounts of several related compounds including griseofulvin (**2c**) and xanthone **3**.<sup>15</sup>



**1a**: R = H  
**1b**: R = Ac



**2a**: R = H; C-5'*R*  
**2b**: R = H; C-5'*S*  
**2c**: R = Cl; C-5'*R*



**3**

Because *Memnoniella* is closely related to *Stachybotrys*,<sup>7</sup> and the latter fungus is known to produce the trichothecene mycotoxins [e.g. trichodermol (**1a**) and trichodermin (**1b**)],<sup>16</sup> the finding of the trichothecenes **1a** and **1b** in *M. echinata*, although previously unreported in *Memnoniella*, is not surprising. However, heretofore, the griseofulvins have been found only in *Penicillium* fungi,<sup>13</sup> and their presence in a distantly related fungus is unexpected. To date, we have not detected the griseofulvins in any of our cultures of *S. atra*.

The *S. atra*-*M. echinata* complex produces a wide variety of biologically active natural products including the trichothecenes, stachybotrylactones, and stachybotrylactams (and related spirodihydrobenzofuran lactones and lactams),<sup>16-21</sup> cyclosporins,<sup>22</sup> and now the griseofulvins. In addition, our preliminary work with highly toxigenic isolates of *S. atra* from the Cleveland study has turned up several additional potent cytotoxins that do not appear to be members of the above classes of antibiotics. Thus, these fungi produce a wide variety of cytotoxins (e.g., trichothecenes) and immunosuppressants (e.g., cyclosporins and stachybotrylactones), the combination of which contributes significantly to the toxicity of these organisms.

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- M. echinata* was grown at ambient temperature on rice (1.5 kg) for 30 days. The culture was dried, ground, and extracted with MeOH to give 38 g of black gum. Twelve g of the crude extract was chromatographed over silica gel (increasing Et<sub>2</sub>O in hexane followed by increasing MeOH in CH<sub>2</sub>Cl<sub>2</sub>) to give, in increasing order of elution, 50 mg of **1b**, 20 mg of **1a**, and 40 mg of **3** in the Et<sub>2</sub>O-hexane fractions and 1 g of a 50–50 mixture of **2a** and **2b** in the 2% MeOH-CH<sub>2</sub>Cl<sub>2</sub> fraction. A small sample (10 mg) of the **2a**, **2b** mixture was subjected to semipreparative reversed-phase HPLC (C<sub>18</sub>, 250 × 10 mm, 45% MeOH in water, flow rate 4 mL/min) to give 5 mg of **2a** (*t*<sub>R</sub> 27.8 min, mp and mmp 181–182 °C)<sup>9</sup> and 5 mg of **2b** (*t*<sub>R</sub> 23.8 min): mp 129–131 °C; [α]<sub>D</sub><sup>20</sup> +28.5° (c 0.2, acetone); IR ν max (CHCl<sub>3</sub>) 1701, 1655, 1618, 1592 cm<sup>-1</sup>; UV λ max (CHCl<sub>3</sub>) (log ε) 248 (4.34), 288 (4.46), 320 (3.86) nm; EIMS *m/z* 318 (100) [M<sup>+</sup>], 287 (57), 250 (34), 138 (65), 106 (40), 69 (21), 51 (22); HREIMS *m/z* found [M<sup>+</sup>] 318.1117, C<sub>17</sub>H<sub>18</sub>O<sub>6</sub>, requires 318.1104; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 0.88 (3 H, d, *J* = 6.6 Hz, H-7), 2.49 (2 H, d, *J* = 8.3 Hz, H-4'), 2.75 (1 H, tq, *J* = 6.6 and 8.3 Hz, H-5'), 3.61 (3 H, s, H-6'), 3.87 (3 H, s, H-10), 3.91 (3 H, s, H-11), 5.54 (1 H, s, H-2'), 6.03 (1 H, d, *J* = 1.5 Hz, H-7), 6.19 (1 H, d, *J* = 1.5 Hz, H-5); <sup>13</sup>C NMR (CDCl<sub>3</sub>) δ 89.3 (C-2), 194.0 (C-3), 158.9 (C-4), 88.8 (C-5), 175.6 (C-6), 93.3 (C-7), 171.0 (C-8), 105.8 (C-9), 56.1 (C-10), 56.0 (C-11), 170.3 (C-1'), 105.3 (C-2'), 197.3 (C-3'), 40.8 (C-4'), 34.9 (C-5'), 56.5 (C-6'), 13.8 (C-7').
- A clear colorless crystal of **2b** with dimensions 0.325 × 0.125 × 0.125 mm was obtained from aqueous methanol. Hydrogen coordinates, thermal parameters, bond distances and angles, and observed and calculated structure factors for **2b** have been deposited with the Cambridge Crystallographic Data Centre and can be obtained, upon request, from Dr. Olga Kennard, University Chemical Laboratory, 12 Union Road, Cambridge CB2 1EZ, UK.
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