7-Chloro-4,6-dimethoxy-1(3H)-isobenzofurane and Basidalin: Antibiotic Secondary Metabolites from *Leucoagaricus carneifolia* Gillet (Basidiomycetes)

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Two antibiotic metabolites were isolated from cultures of *Leucoagaricus carneifolia*. Their structures were elucidated by spectroscopic methods. The first compound, 7-chloro-4,6-dimethoxy-1(3H)-isobenzofurane (1) had to our knowledge not been described from natural sources whereas the second, basidalin (2), is a known metabolite of *L. naucina* (H. Iinuma et al., 1983). 1 exhibits antibiotic activities with minimal inhibitory concentrations of 20 μg/ml against *Botrytis cinerea*, the most sensitive microorganism.

Introduction

The genus *Leucoagaricus* (Loquin) Sing, is most closely related to the genera *Macrolepiota* and *Leucocoprinus*. It is almost cosmopolitan, most species, however, have been described from America and Africa (R. Singer, 1986). In the course of a screening for new secondary metabolites from basidiomycetes the European species *Leucoagaricus carneifolia* was found to produce two antibiotic and cytotoxic compounds. In the following we wish to describe the fermentation, isolation, structural elucidation and biological characterization of these metabolites which were identified as 7-chloro-4,6-dimethoxy-1(3H)-isobenzofurane (1) and basidalin (2).

Materials and Methods

*Leucoagaricus carneifolia* strain 90352

Mycelial cultures of *L. carneifolia* were obtained from spore prints of fruiting bodies collected in Mölschbach, Germany. Herbarium specimen and mycelial cultures are deposited in the collection of strains, LB Biotechnologie, University of Kaiserslautern.

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For maintenance on agar slants and submerged cultivation L. carneifolia was grown in a yeast extract – malt extract – glucose (YMG) medium composed of g/l: yeast extract 4, malt extract 10 and glucose 4. A 250 ml culture of L. carneifolia grown for 9 days was used to inoculate 20 l of the same medium in a Biolafitte C6 fermentation apparatus. After 16–18 days of fermentation (25 °C, 150 r.p.m., 3.3 l air/min) the mycelia were separated from the culture fluid and discarded. 1 and 2 were adsorbed from the filtrate (15 l) on HP21-resin (Mitsubishi) and eluted with methanol. The solvent was removed under reduced pressure and the oily residue (1.5 g) was applied to a column (silica gel Merck 60; 4x50 cm). 1 and 2 were eluted with cyclohexane–ethyl acetate (8:2; 1) and (6:4; 2). Yields: 150 mg of 1 and 400 mg of 2.

**Results and Discussion**

**Fermentation and isolation**

White powder, m.p. 164.1 °C, soluble in methanol, acetone, retention time 7.2 min [LiChrospher 60 RP-select B (125x4 mm), H2O–MeCN gradient, 2–100% MeCN in 9 min, flow 1.2 ml/min, detection: UV absorption at 210 nm]; UV (MeOH) λ_max nm (ε) 217 (42,000), 257 (11,300), 306 (3600); IR (KBr) cm⁻¹ 3430 (w), 2956 (w), 2985 (w), 1757 (s), 1618 (m), 1577 (m), 1478 (w), 1414 (s), 1341 (s), 1120 (s), 1038 (s), 768 (m); EI-MS, MS, m/e 228 (M+, calculated for C10H8O4Cl, 228.63). ¹H NMR (CDCl₃, 400 MHz) δ 7.16 (1H, Ar-H), 5.41 (2H, CH₂), 4.02 (2H, CH₂), 3.97 (3H, O-CH₃); ¹³C NMR (CDCl₃, 100.61 MHz) δ 170.4 (C=O), 157.9 and 151.5 (2× s, C-OME), 128.9 (C-8), 125.45 (C-9), 121.5 (C=Cl), 102.1 (2H, CH), 67.9 (CH₂), 59.8 and 56.9 (2× q, O-CH₃) ppm; Elementary analysis (calculated for C₁₀H₈O₄Cl: C 52.5%, H 4.0%; found: C 52.2%, H 4.2%).

**Biological assays**

The antimicrobial spectra were determined in the serial dilution assay. [YMG medium, 27 °C for fungi; Nutrient Broth (Difco), 37 °C for bacteria] (Kupka et al., 1979). The cytotoxicity against L 1210 cells (mouse) was determined as described previously (Leonhardt et al., 1987). 10⁵/ml L 1210 cells (ATCC CCL219) were incubated in F12 medium containing 1.5% horse serum with or without antibiotic. After 1 and 2 days the cells were counted using a microscope.

**Structure elucidation**

### 7-Chloro-4,6-dimethoxy-1(3H)-isobenzofurane (1)

White powder, m.p. 164.1 °C, soluble in methanol, acetone, retention time 7.2 min [LiChrospher 60 RP-select B (125x4 mm), H₂O–MeCN gradient, 2–100% MeCN in 9 min, flow 1.2 ml/min, detection: UV absorption at 210 nm]; UV (MeOH) λ_max nm (ε) 217 (42,000), 257 (11,300), 306 (3600); IR (KBr) cm⁻¹ 3430 (w), 2956 (w), 2985 (w), 1757 (s), 1618 (m), 1577 (m), 1478 (w), 1414 (s), 1341 (s), 1120 (s), 1038 (s), 768 (m); EI-MS, MS, m/e 228 (M+, calculated for C₁₀H₈O₄Cl, 228.63). ¹H NMR (CDCl₃, 400 MHz) δ 7.16 (1H, Ar-H), 5.41 (2H, CH₂), 4.02 (2H, CH₂), 3.97 (3H, O-CH₃); ¹³C NMR (CDCl₃, 100.61 MHz) δ 170.4 (C=O), 157.9 and 151.5 (2× s, C-OME), 128.9 (C-8), 125.45 (C-9), 121.5 (C=Cl), 102.1 (2H, CH), 67.9 (CH₂), 59.8 and 56.9 (2× q, O-CH₃) ppm; Elementary analysis (calculated for C₁₀H₈O₄Cl: C 52.5%, H 4.0%; found: C 52.2%, H 4.2%).
Table I. $^{13}$C NMR spectral data of 7-chloro-4,6-dimethoxy-1(3H)-isobenzofurane (1).

<table>
<thead>
<tr>
<th>C-Atom</th>
<th>$^{13}$C [ppm]</th>
<th>$^{1}$J$_{HC}$ [Hz]</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>170.4 (s)</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>67.9 (t)</td>
<td>145</td>
</tr>
<tr>
<td>4</td>
<td>151.5 (s)</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>102.1 (d)</td>
<td>168</td>
</tr>
<tr>
<td>6</td>
<td>157.8 (s)</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>121.5 (s)</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>128.9 (s)</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>125.5 (s)</td>
<td></td>
</tr>
<tr>
<td>10, 11</td>
<td>57.0 (q), 59.9 (q)</td>
<td>145</td>
</tr>
</tbody>
</table>

The $CH_2$ group (C-3) appeared at 67.9 ppm ($^{1}$J$_{C-H}$ = 145 Hz). The signal at 170.4 ppm was assigned to C-1.

The structure 1 deduced from our spectroscopic data is isomeric to 4-chloro-5,7-dimethoxy-1(3H)-isobenzofurane, a compound synthesized previously by Mirrington et al. (1966). A direct comparison of their $^1$H NMR data yielded additional proof for structure 1. Phthalides are among the more common natural compounds and a variety of biological activities have been reported for these metabolites [W. B. Turner and D. C. Aldrich, 1983, Dictionary of Natural Products (J. Buckingham, ed.), 1992]. Mycophenolic acid, one of the first known secondary metabolites isolated from fungi, is a phthalide derivative (for review see V. Betina, 1989).

Basidalin [4-amino-5-(formylmethylene)-2(5H)-furanone] was identified (T. Huff, 1993) by direct comparison with the spectral data published by Iinuma et al. (1983).

### Biological activities

The antimicrobial activities of 1 and 2 in the serial dilution assay are shown in Table II. 1 exhibits selective activity against Botrytis cinerea with a minimal inhibitory concentration (MIC) of 20 µg/ml. All other fungi and bacteria were not inhibited by 100 µg/ml. No cytotoxic activities against L 1210 cells (mouse, lymphocytic leukemia) were detected. 2 shows weak antibiotic activities towards B. brevis, M. luteus, Streptomyces sp. ATCC 23836, and B. cinerea. Iinuma et al. (1983) had reported weak antibacterial activity towards Aeromonas salminicola and Vibrio anguillarum. The observed cytotoxic activities of basidalin (1 µm/ml, L 1210 cells) are in good agreement with the data of Iinuma et al. (1983).

### Acknowledgements

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