Prenisatin (5-(3-Methyl-2-butenyl)-indole-2,3-dione): an Antifungal Isatin Derivative from Chaetomium globosum

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A novel metabolite, named prenisatin, was isolated from submerged fermentations of Chaetomium globosum Kunze:Fr. By means of NMR, MS, IR and UV analyses, the structure of prenisatin was determined to be 5-(3-methyl-2-butenyl)-indole-2,3-dione (5-prenylisatin). Prenisatin exhibits in vitro growth inhibitory activity against Botrytis cinerea.

Growing concern about the environmental impact of the chemicals used for controlling pests in crops, as well as the frequently observed resistance to currently used pesticides, warrant a continuous and intensified search for new plant protection agents. Micro-organisms have proven to be a rich source of structurally diverse metabolites, and our current research is focussed on the discovery of novel antifungal compounds from microbial sources. In this context the fungus Chaetomium globosum Kunze:Fr, isolated from a soil sample collected under a tree in Egypt, and propagated in submerged fermentations, was found to produce a substance which in vitro inhibits the growth of phytopathogenic fungi, especially Botrytis cinerea (grey mould). The structure of the active component, an orange solid designated prenisatin, was elucidated by means of NMR, MS, IR and UV analyses and determined to be 5-(3-methyl-2-butenyl)-indole-2,3-dione (5-prenylisatin) (1). Along with prenisatin, a purple pigment was isolated and identified as cochliodinol (2), previously obtained from C. globosum and C. cochliodes, upon comparison of spectral and chemical data with literature values.1 We report on the production, isolation and structure elucidation of prenisatin.

Isolation and structure

Chaetomium globosum Kunze:Fr was fermented in shake-flask cultures on a soy bean-based medium. The active principle present in the EtOAc extract of homogenized cultures was located by fractionating the extract by reversed-phase HPLC and testing each fraction for activity against the target organism. The activity was related to an orange component which was purified by silica gel chromatography followed by reversed-phase HPLC, as described in the Experimental section. From 1 of culture broth prenisatin (1) (5.2 mg) and cochliodinol (6.5 mg) were obtained as orange and purple solids, respectively.

The 1H NMR spectrum (CDCl3) signalled the presence of three protons in a 1,3,4-arrangement in an aromatic ring carrying a 3-methyl-2-butenyl (prenyl) moiety as one of the substituents. The well resolved 1H NMR

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spectrum allowed the \(^1\text{H},^1\text{H}\)-connectivities to be traced out by a series of homo-decoupling experiments. One exchangeable proton appeared as a broad hump centered around \(\delta 4.3\).

\(^{13}\text{C}\) NMR spectroscopy, including DEPT experiments, demonstrated the presence of two methyl groups (\(\delta 17.82\) and 25.73), one methylene group (\(\delta 33.38\)), four proton-bearing sp\(^2\) carbon atoms (\(\delta 112.28, 121.75, 125.36\) and 138.67) and six quaternary carbon atoms in the aromatic/carbonyl region (\(\delta 118.14, 134.03, 137.99, 147.31, 159.76\) and 183.37).

The EI-MS exhibited a molecular ion at \(m/z\) 215 (base peak) which, taking the NMR-established content of 13 carbon atoms and 13 protons into account, suggested \(\text{C}_{13}\text{H}_{11}\text{NO}_3\) as the molecular formula for prenisatin, as confirmed by high-resolution measurements.

The HMBC spectrum, recorded without \(^{13}\text{C}\)-decoupling in order to observe one-bond \(^1\text{H},{^13}\text{C}\)-couplings (\(J_{\text{CH}}\)), served to assign the proton-bearing carbon atoms, and showed \(J_{\text{CH}}\) for the aromatic CH groups to fall within the range 159–164 Hz, typical for six-membered aromatic systems. \(^1\text{H},{^13}\text{C}\) long-range correlations observed in the HMBC spectrum (optimized for \(J_{\text{CH}} = 6\) Hz) allowed connection of the partial structures. Thus, the position of the isopentenyl side chain remains unambiguously determined by the correlations observed from the side-chain methylene group (\(\text{H}_2\text{C}-\text{I}^\prime\)) to C-4, C-5 and C-6, and the characteristic three-bond correlations in the aromatic system served to assign the quaternary ring carbon atoms (C-3a, C-5 and C-7a). Additionally, the observed correlation from H-4 to the carbonyl group at \(\delta 183.37\) (C-3) establishes the C-3,C-3a connection. The assigned \(^1\text{H}\) and \(^{13}\text{C}\) NMR data for prenisatin (1) are listed in Table 1, along with the corresponding \(^{13}\text{C}\) NMR shifts reported for 5-methylisatin (5-MI). Long-range \(^1\text{H},{^13}\text{C}\)-correlations observed in the HMBC-spectrum are indicated in Fig. 1.

Convincing agreement between the data sets results from interchange of the assignments reported\(^2\) for C-4 and C-6 in 5-MI. The proposed structure (1) for prenisatin receives further support by comparing UV and IR data for 1 and the parent isatin. Thus, the recorded UV absorption maxima for 1 (\(\lambda_{\text{max}}/\text{nm} (\log e)\) (MeOH) 247 (4.28), 299 (3.59) and 422 (2.82)) are similar but, as expected, slightly red-shifted relative to those of isatin under identical conditions [242 (4.32), 296 (3.49) and 410 (2.82)]. The IR spectrum (in KBr) of 1 is dominated by strong absorptions at 1741 and 1728 cm\(^{-1}\), attributable to C=O stretching bands for the dione moiety, and strong aromatic bands are observed at 1621 and 1490 cm\(^{-1}\). Isatin exhibits corresponding bands of similar intensity at 1750, 1726, 1616 and 1460 cm\(^{-1}\).

**Discussion**

A vast number of isatin (indole-2,3-dione) derivatives have been prepared by chemical synthesis\(^2\) and evaluated with respect to biological activity, but only a few have been reported from natural sources. Isatin, the parent compound, has been described as a defensive metabolite of the marine bacteria *Alteromonas* sp.,\(^3\) and identified as the endogenous monoamine oxidase inhibitor, tribulin, detected in normal human urine.\(^4\) A close analogue of prenisatin, the positional isomer, 6-(3-methyl-2-butenyl)-indole-2,3-dione, has been described from *Streptomyces albicus*,\(^5\) and 5-chloro-6-methoxy-1-methylindole-2,3-dione as a metabolic product of *Micromonospora carboxonae*.\(^6\) Finally, three phyllopetalinsates, Melosatin A, B and C, have been isolated from the tumorigenic plant species *Melochia tomentosa*.\(^7,8\)

**Experimental**

*General.* IR spectra were recorded (KBr) on a Perkin-Elmer 1720 instrument. High-resolution mass spectra were recorded at 70 eV ionization potential on a VG70–250SE instrument (VG Analytical). EI spectra are presented as \(m/z\) (% rel. int.). NMR spectra were acquired in chloroform-d at 297 K on a Bruker AC300P instrument equipped with a \(^1\text{H},{^{13}}\text{C}\) dual probe operating at 300.13 and 75.47 MHz for \(^1\text{H}\) and \(^{13}\text{C}\), respectively. Solvent peaks [7.27 ppm (\(^1\text{H}\)) and 77.00 ppm (\(^{13}\text{C}\))] were used for scaling.

Preparative HPLC separations were performed using Gilson 305/306 master/slave pumps and a Pye Unicam

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Table 1. \(^1\text{H}\) and \(^{13}\text{C}\) NMR data for prenisatin (1) and \(^{13}\text{C}\) NMR data for 5-MI.\(^2\)

<table>
<thead>
<tr>
<th>No.</th>
<th>(^1\text{H}) (1H, br)</th>
<th>(^{13}\text{C})</th>
<th>5-MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.3 (1H, br)</td>
<td>159.76</td>
<td>159.5</td>
</tr>
<tr>
<td>2</td>
<td>–</td>
<td>183.37</td>
<td>184.6</td>
</tr>
<tr>
<td>3a</td>
<td>–</td>
<td>118.14</td>
<td>117.8</td>
</tr>
<tr>
<td>4</td>
<td>7.40 (1H, s, br)</td>
<td>125.36</td>
<td>124.8*</td>
</tr>
<tr>
<td>5</td>
<td>–</td>
<td>137.99</td>
<td>132.0</td>
</tr>
<tr>
<td>6</td>
<td>7.36 (1H, dd, 8.0/1.7)</td>
<td>138.67</td>
<td>138.8*</td>
</tr>
<tr>
<td>7</td>
<td>6.84 (1H, d, 8.0)</td>
<td>112.28</td>
<td>112.1</td>
</tr>
<tr>
<td>7a</td>
<td>–</td>
<td>147.31</td>
<td>148.5</td>
</tr>
<tr>
<td>1’</td>
<td>3.29 (2H, d, 7.4)</td>
<td>33.38</td>
<td>–</td>
</tr>
<tr>
<td>2’</td>
<td>5.29 (1H, m)</td>
<td>121.75</td>
<td>–</td>
</tr>
<tr>
<td>3’</td>
<td>–</td>
<td>134.03</td>
<td>–</td>
</tr>
<tr>
<td>4’</td>
<td>1.71 (3H, s, br)</td>
<td>25.73</td>
<td>–</td>
</tr>
<tr>
<td>5’</td>
<td>1.76 (3H, s, br)</td>
<td>17.82</td>
<td>–</td>
</tr>
</tbody>
</table>

* Assignments reversed in comparison with the literature.\(^2\)
LC3 UV detector. The column was 250 × 20 mm ODS 10 μm reversed-phase (DuPont) eluted with gradients of acetonitrile (solvent B) in deionized water (solvent A) at a flow rate of 15 ml/min.

Bioassays. Growth inhibitory activity against Botrytis cinerea was measured in an agar diffusion assay essentially as described previously.9

Cultivation and fermentation. The fungus Chaetomium globosum Kunze:Fr (NN003293, Novo Nordisk Strain Collection, Copenhagen) was cultivated on agar slants [0.4% yeast extract, 0.1% KH₂PO₄, 0.05% MgSO₄·7H₂O, 1.5% glucose and 2% Bacto™ (Difco) in 1 l of distilled water] for 7–14 days at 26°C. The entire mixture of young perithecia, ascospores and mycelium, prepared by washing the slants with sterile, distilled water containing 0.1% Tween 20, was used to inoculate the shake flasks. The flasks were 500 ml Erlenmeyer flasks with 2 baffles, each containing 100 ml of growth media prepared as follows: potato flour (75 g), Ban 800 MG (0.075 g) and tap water (800 ml) were mixed and heated at 60–70°C for 10 min and at 70°C for 20 min and then quickly brought to boiling point. To this mixture was added soy bean meal (40 g), Na₂HPO₄·12H₂O (9 g), KH₂PO₄ (1.5 g), Pluronic 100% (0.1 ml) and tapwater to a total volume of 1 l. The medium was homogenized and sterilized at 121°C for 40 min. The pH after sterilization was 6.46.

Purification and properties. Approximately 1 l (10 shake flasks) of culture broth was homogenized and extracted with 2 × 500 ml of EtOAc. After drying, performed by cooling the extract to −18°C and removing the separated ice by filtration, the solvent was evaporated in vacuo. The residue was dissolved in heptane–EtOAc and applied to a silica gel column (240 × 40 mm, Merck Si60, 0.063–0.200 mm packed in heptane) and eluted with the following step gradient: heptane (600 ml), heptane–EtOAc 7:3 (450 ml), heptane–EtOAc 2:8 (600 ml), EtOAc (450 ml), EtOAc–MeOH 3:1 (300 ml), EtOAc–MeOH 1:1 (300 ml) and MeOH (600 ml).

Cochlidiolin (2) eluted with heptane–EtOAc 2:8 and was further purified by preparative reversed-phase HPLC (88% B = >75% B over 30 min). 2 (6.5 mg l⁻¹ fermentation broth) was identified by comparing its spectroscopic data (UV, IR, MS, NMR) with literature values.1

Prenisatin was present in the fractions eluted with mixtures of EtOAc–MeOH, and likewise purified by HPLC (40% B/55% B over 30 min) to afford the pure compound as an orange solid (5.2 mg l⁻¹): HREI-MS: 215 (100, M⁺), found 215.0932, calc. for C₁₃H₁₆NO₂ 215.0946, 200 (4, [M–CH₃]⁺), 187 (62, [M–CO]⁺), found 187.0996, calc. for C₁₂H₁₃NO 187.0979, 172 (36, [M–CO–CH₃]⁺), found 172.0759, calc. for C₁₁H₁₂NO 172.0762, 158 (6), 144(27), 132(6), 129(6), 117(6), 115(6), 91(5), and 77(5). IR in KBr (ν max/cm⁻¹): 3262 (NH, br) 1741 (C=O), 1728 (C=O), 1621 (Ar), and 1490 (Ar). UV data were presented in the text above. 1H and 13C NMR data are shown in Table 1.

References

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