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

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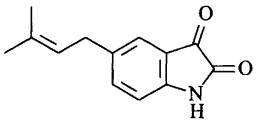
A novel metabolite, named prenisatin, was isolated from submerged fermentations of *Chaetomium globosum* Kunze: Fr. By means of NMR, MS, IR and UV analyses, the structure of prenisatin was determined to be 5-(3-methyl-2-butenyl)-indole-2,3-dione (5-prenylisatin). Prenisatin exhibits *in vitro* growth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



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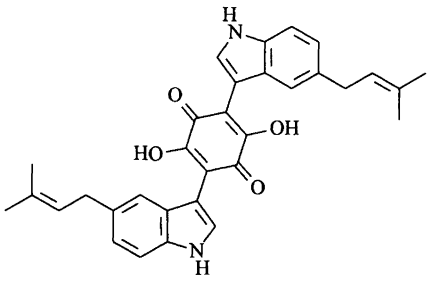

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Isolation and structure

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 l of culture broth prenisatin (**1**) (5.2 mg) and cochliodinol (6.5 mg) were obtained as orange and purple solids, respectively.

The ^{1}H NMR spectrum ($CDCl_3$) 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 ^{1}H NMR

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spectrum allowed the $^1H,^1H$-connectivities to be traced out by a series of homo-decoupling experiments. One exchangeable proton appeared as a broad hump centered around δ 4.3.

^{13}C NMR spectroscopy, including DEPT experiments, demonstrated the presence of two methyl groups (δ 17.82 and 25.73), one methylene group (δ 33.38), four proton-bearing sp^2 carbon atoms (δ 112.28, 121.75, 125.36 and 138.67) and six quaternary carbon atoms in the aromatic/carbonyl region (δ 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 $C_{13}H_{13}NO_2$ as the molecular formula for prenisatin, as confirmed by high-resolution measurements.

The HMQC spectrum, recorded without ^{13}C-decoupling in order to observe one-bond $^1H,^{13}C$-couplings ($^1J_{CH}$), served to assign the proton-bearing carbon atoms, and showed $^1J_{CH}$ for the aromatic CH groups to fall within the range 159–164 Hz, typical for six-membered aromatic systems. $^1H,^{13}C$ long-range correlations observed in the HMBC spectrum (optimized for J_{CH} = 6 Hz) allowed connection of the partial structures. Thus, the position of the isopentenyl side chain is unambiguously determined by the correlations observed from the side-chain methylene group (H_2C-1′) 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 δ 183.37 (C-3) establishes the C-3,C-3a connection. The assigned ^1H and ^{13}C NMR data for prenisatin (**1**) are listed in Table 1, along with the corresponding ^{13}C NMR shifts reported for 5-methylisatin (5-MI).[2] Long-range $^1H,^{13}C$-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** [λ_{max}/nm(log ε) (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}.

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

No.	^1H	^{13}C	5-MI
1	4.3 (1H, br)		
2	–	159.76	159.5
3	–	183.37	184.6
3a	–	118.14	117.8
4	7.40 (1H, s, br)	125.36	124.8[a]
5	–	137.99	132.0
6	7.36 (1H, dd, 8.0/1.7)	138.67	138.8[a]
7	6.84 (1H, d, 8.0)	112.28	112.1
7a	–	147.31	148.5
1′	3.29 (2H, d, 7.4)	33.38	–
2′	5.25 (1H, m)	121.75	–
3′	–	134.03	–
4′	1.71 (3H, s, br)	25.73	–
5′	1.76 (3H, s, br)	17.82	–

[a] Assignments reversed in comparison with the literature.[2]

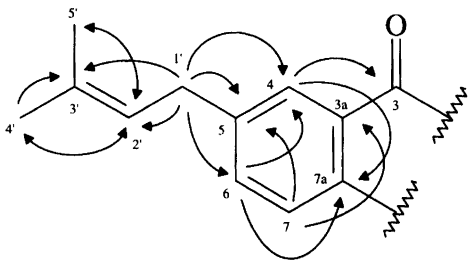

Fig. 1. Long-range ^1H, ^{13}C correlations observed in the HMBC spectrum indicated by arrows.

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 albus*,[5] and 5-chloro-6-methoxy-1-methylindole-2,3-dione as a metabolic product of *Micromonospora carbonaceae*.[6] Finally, three phenylpentylisatines, 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 ^1H–^{13}C dual probe operating at 300.13 and 75.47 MHz for ^1H and ^{13}C, respectively. Solvent peaks [7.27 ppm (^1H) and 77.00 ppm (^{13}C)] were used for scaling.

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

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% KH_2PO_4, 0.05% $MgSO_4 \cdot 7H_2O$, 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), $Na_2HPO_4 \cdot 12H_2O$ (9 g), KH_2PO_4 (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). Cochliodinol (**2**) eluted with heptane–EtOAc 2:8 and was further purified by preparative reversed-phase HPLC (68% 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 $C_{13}H_{13}NO_2$ 215.0946), 200 (4, $[M\text{-}CH_3]^+$), 187 (62, $[M\text{-}CO]^+$, found 187.0996, calc. for $C_{12}H_{13}NO$ 187.0997), 172 (36, $[M\text{-}CO\text{-}CH_3]^+$, found 172.0759, calc. for $C_{11}H_{10}NO$ 172.0762), 158(6), 144(27), 132(6), 129(6), 117(6), 115(6), 91(5), and 77(5). IR in KBr (λ_{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 ^{13}C NMR data are shown in Table 1.

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