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Generation of Indole Alkaloids in the Human-Pathogenic Fungus *Exophiala dermatitidis*

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Dedicated to Professor Horst Kessler on the occasion of his 70th birthday

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Exophialin, a previously unknown indole alkaloid, was isolated from the human-pathogenic fungus *Exophiala dermatitidis* upon cultivation on a medium containing tryptophan as sole nitrogen source. Its structure has been elucidated by 1D and 2D NMR experiments and mass spectrometry. When the mutant strain Mel-1 was used in place of the wild-type strain, the new indole alkaloid 8-hydroxyexophialin was generated instead of exophialin. This result indicates that the aromatic moiety of exophialin originates from 2-hydroxyjuglone, a shunt product of the 1,8-dihydroxynaphthalene melanin biosynthesis, which is only produced by the wild-type strain, but not by the mutant. In contrast, the mutant accumulates flavioline, which acts as precursor leading to the generation of 8-hydroxyexophialin instead of exophialin.

Introduction

Exophiala dermatitidis (Kano) de Hoog (Synonym: *Wangiella dermatitidis*) is a black yeast-like fungus[1] causing cutaneous and subcutaneous phaeohyphomycoses in both immunocompetent and immunocompromised patients.[2] *E. dermatitidis* serves as the model of choice for discovering cell wall-related virulence determinants.[3] The biosynthesis of 1,8-dihydroxynaphthalene melanin in particular has been studied in detail in *E. dermatitidis*,[4] since this compound contributes to its pathogenicity.[5] Nevertheless, apart from some aromatic pentaketides involved in the biosynthesis of 1,8-dihydroxynaphthalene melanin,[4] there are no reports on the occurrence of other secondary metabolites in *E. dermatitidis* despite the possibility that these might be important for the pathogenicity. A number of indole alkaloids have been isolated from the human-pathogenic lipophilic yeast *Malassezia furfur*, one of the causative agents of pityriasis versicolor.[6] Several of these indole alkaloids are suspected of contributing to the pathogenicity of *M. furfur*. We decided therfore to extend the screening for indole alkaloids to *E. dermatitidis* including different mutants[4] deficient in the biosynthesis of 1,8-dihydroxynaphthalene melanin.

In order to enhance the production of indole alkaloids, *E. dermatitidis* was grown on a solid medium which contained tryptophan as sole nitrogen source. This method has already been applied successfully to stimulation of the production of indole alkaloids in *M. furfur*.[6] By this means, exophialin (**2**) has been isolated from the wild-type strain of *E. dermatitidis*, while 8-hydroxyexophialin (**1**) has been isolated from the mutant Mel-1 of *E. dermatitidis* which is deficient in the biosynthesis of 1,8-dihydroxynaphthalene melanin, thus enriching the aromatic polyketide flavioline (**7**) (Figure 1). The origin of the new alkaloids and of the known indole alkaloid pityriacitrin (**3**)[7] has also been investigated.

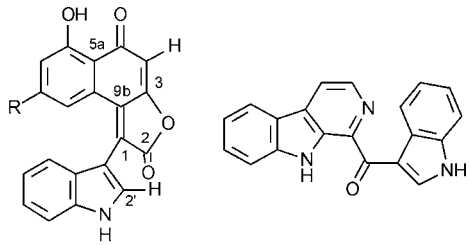

Figure 1. Indole alkaloids from *E. dermatitidis* and *E. dermatitidis* Mel-1.

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Results and Discussion

The indole alkaloids were extracted with ethyl acetate from solid cultures of *E. dermatitidis* grown on a medium containing glucose, Tween 80 and tryptophan as the sole nitrogen source. The crude alkaloids were subsequently purified on Sephadex LH 20, on preparative silica gel 60 thin layer plates and by preparative HPLC on an RP-8 column. 200 plates on which *E. dermatitidis* have been grown yielded only 0.4 mg of exophialin (**2**). However, 1.8 mg of 8-hydroxyexophialin (**1**) was isolated from 200 plates on which the mutant *E. dermatitidis* Mel-1 had been grown. We therefore decided to elucidate the structure of **1** first.

The red alkaloid 8-hydroxyexophialin (**1**) exhibits absorption maxima at λ = 218, 274, 318 and 501 nm. The $[M + H]^+$ ion at m/z = 346.0709 in the LC-HRMS (APCI) corresponds to the molecular formula $C_{20}H_{11}NO_5$ indicating the presence of sixteen double bond equivalents. The carbon content of the molecular formula is corroborated by the ^{13}C NMR spectrum which shows twenty carbon resonances between δ_C = 104.9 and 190.6 ppm (Table 1). The ^1H NMR spectrum reveals eight protons in the aromatic region. According to the COSY experiment, the signals at δ_H = 7.00 (d), 7.15 (dd), 7.28 (dd), and 7.57 (d) ppm belong to an *ortho* substituted aromatic ring which is part of an indole ring. Considering the correlations in the HMBC and HSQC, the carbons and the protons of the indole ring system can be assigned unambiguously, and the singlet at δ_H = 8.03 ppm represents 2′-H of the indole ring. This proton exhibits – apart from correlations to several carbons of the indole ring – three correlations to the carbons at δ_C = 125.7, 132.3 and 169.7 ppm (Figure 2 and Table 1).

Table 1. NMR spectroscopic data of 8-hydroxyexophialin (**1**) and exophialin (**2**).

	8-Hydroxyexophialin (**1**)		Exophialin (**2**)	
#	δ_C[a]	δ_H (mult., J in Hz)[b]	δ_C[c]	δ_H (mult.) [d]
1	125.7			
2	169.7			
3	163.5			
4	104.9	6.18 (s)	104.9	6.36 (s)
5	190.6			
5a	108.6			
6	166.5			
7	105.9	6.32 (d, 2.0)	120.9	7.04 (d, 8.5)
8	165.8		135.4	7.33 (dd, 8.5, 7.9)
9	111.6	6.53 (d, 2.0)	121.5	7.02 (d, 7.9)
9a	131.9			
9b	132.3			
2′	132.6	8.03 (s)	132.9	8.09 (s)
3′	106.4			
3a′	125.2			
4′	123.5	7.00 (d, 8.0)	123.4	6.93 (d, 7.9)
5′	122.0	7.15 (dd, 8.0, 7.0)	121.6	7.13 (dd, 7.9, 7.0)
6′	124.1	7.28 (dd, 8.2, 7.0)	123.9	7.30 (dd, 8.2, 7.0)
7′	113.7	7.57 (d, 8.2)	113.6	7.59 (d, 8.2)
7a′	138.6			

[a] 151 MHz, CD_3OD, 300 K. [b] 600 MHz, CD_3OD, 300 K. [c] 151 MHz, CD_3OD, 277 K, quaternary signals missing, CH signals derived from the HSQC. [d] 600 MHz, CD_3OD, 277 K.

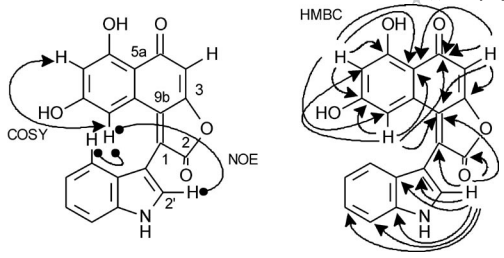


Figure 2. Selected COSY (↔), NOE (·–·) and HMBC (→) correlations of 8-hydroxyexophialin (**1**).

Two doublets at δ_H = 6.32 and 6.53 ppm are part of another aromatic ring. Due to the small coupling constant of J_{HH} = 2.0 Hz, the protons must be located *meta* to each other. The shift values δ_H = 6.32 and 6.53 ppm indicate the presence of hydroxy groups adjacent to these aromatic protons. Taking the correlations in the HSQC and in the HMBC into account, the carbons and protons are assigned to this part of the structure. Two hydroxy groups are attached to the carbons at δ_C = 165.8 and 166.5 ppm which are located *ortho* to the proton at δ_H = 6.32 ppm. Two correlations from the proton at δ_H = 6.53 and that at δ_H = 6.32 ppm to carbons at δ_C = 132.3 and 190.6 ppm are assigned to carbons adjacent to the *meta* substituted aromatic ring. The resonance at δ_C = 190.6 ppm represents a C=O group on the basis of its shift value. It is located at position 5, since $^4J_{CH}$ couplings are recognized both from the proton at δ_H = 6.32 and at δ = 6.53 ppm. A correlation from 2′-H of the indole ring to the carbon at δ_C = 132.3 ppm indicates that the indole ring is attached via this carbon to the *meta* substituted aromatic ring (Figure 2).

The singlet at δ_H = 6.18 ppm in the ^1H NMR is located as part of a quinone methide moiety in *ortho* position to the C=O group and the carbon at δ_C = 163.5 ppm, carrying an OR group, since it shows strong correlations to the carbons at δ_C = 108.6, 132.3 and 163.5 ppm. The chemical shifts of 4-H, 7-H and 9-H of **1** are in good agreement with those of structurally related synthetic naphthoquinone methides.[8] Considering the molecular formula and the double bond equivalents, the carbon at δ_C = 169.7 ppm has to be placed at C-2 forming a lactone ring to C-3 (Figure 2). The structure of 8-hydroxyexophialin (**1**) as deduced above is corroborated by NOE correlations between 2′-H and 9-H and between 4′-H and 9-H.

The UV/Vis spectrum of the red pigment **2** isolated from the wild-type strain CBS 207.35 of *E. dermatitidis* which we named exophialin exhibits absorption maxima at λ = 204, 270, 308 and 525 nm resembling those of **1**. The $[M + H]^+$ ion at m/z = 330.0761 in the LC-HRMS (APCI) corresponds to the molecular formula $C_{20}H_{11}NO_4$ indicating that **2** contains one oxygen less than **1**. This is in agreement with the ^1H NMR which exhibits nine aromatic protons instead of eight (Table 1). These results indicate that **1** and **2** are closely related to each other differing only in the replacement of one OH group in **2** by a hydrogen atom. An analysis of the spin systems occurring in the ^1H NMR and in the COSY spectrum are in agreement with this deduc-

tion. The resonances at δ_H = 6.93 (d), 7.13 (dd), 7.30 (dd) and 7.59 (d) ppm form a spin system indicating the presence of an *ortho* substituted moiety analogous to that in **1**. Therefore, this spin system has been assigned to an indole moiety. In addition a singlet at δ_H = 8.09 ppm is present in the ^{1}H NMR which has been identified as 2′-H of the indole rest. The proton resonance of 4-H, the proton of the quinone methide moiety occurs in **2** at δ_H = 6.36 ppm. All proton resonances of exophialin (**2**) discussed so far are in close agreement to the corresponding proton resonances of **1** indicating that both compounds are structurally identical in those areas which are in the neighbourhood of these protons. In contrast, the spin system consisting of the protons at δ_H = 7.04 (d), 7.33 (dd) and 7.02 (d) ppm is only present in **2** indicating that the hydroxy group at C-8 of **1** is replaced by a hydrogen atom in exophialin (**2**). A NOE between 4′-H (δ_H = 6.93) and 9-H (δ_H = 7.02 ppm) supports this conclusion (Figure 3). Although the quantity of exophialin (**2**) isolated was too low to record an HMBC in order to obtain complete ^{13}C NMR spectroscopic data of the compound, it was possible to aquire an HSQC. The ^{13}C NMR shifts of the non-quaternary carbon atoms are in perfect agreement with the proposed structure (Table 1).

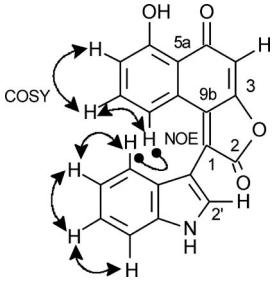
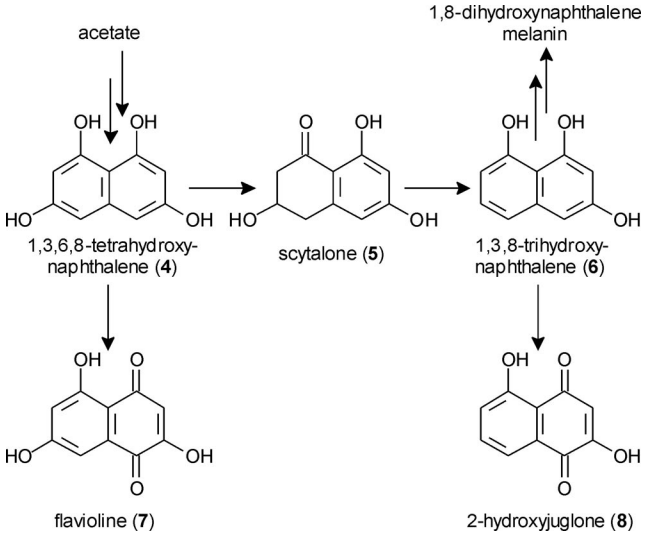


Figure 3. Selected COSY (↔) and NOE (·–·) correlations of exophialin (**2**).

The wild-type and the mutant of *E. dermatitidis* produce different compounds indicating that **1** and **2** are likely to be partially generated from biosynthetic precursors which are involved in the biosynthesis of 1,8-dihydroxynaphthalene melanin, which originates from acetate via 1,3,6,8-tetrahydroxynaphthalene (**4**). The mutant Mel-1 deficient in the biosynthesis of 1,8-dihydroxynaphthalene melanin accumulates the aromatic pentaketide scytalone (**5**), a precursor of 1,8-dihydroxynaphthalene melanin, and flavioline (**7**), a shunt product of this biosynthetic pathway, because the enzyme which converts scytalone to 1,3,8-trihydroxynaphthalene (**6**) is inactive in the mutant (Scheme 1).[9]

The generation of 8-hydroxyexophialin (**1**) in the mutant Mel-1 instead of exophialin (**2**) can be explained unambiguously according to Scheme 1, since **1** might be generated by condensation of flavioline (**7**) with 3-indoleacetic acid or 3-indolepyruvic acid. In contrast, the wild-type strain mainly generates expohialin (**2**) together with small quantities of 8-hydroxyexophialin (**1**). This can be attributed to the fact that flavioline (**7**) is not enriched in the wild-type, instead 2-hydroxyjuglone (**8**), a shunt product of the melanin biosynthesis, is present which obviously reacts instead of **7** with



Scheme 1. Biosynthesis of 1,8-dihydroxynaphthalene melanin in *E. dermatitidis*.[9]

3-indoleacetic acid or 3-indolepyruvic acid, thus generating exophialin (**2**). The yield of **1** in the mutant is higher than the yield of **2** in the wild-type, probably because the aromatic polyketides are used mainly for the biosynthesis of 1,8-dihydroxynaphthalene melanin and are therefore only present in small quantities in the wild-type.

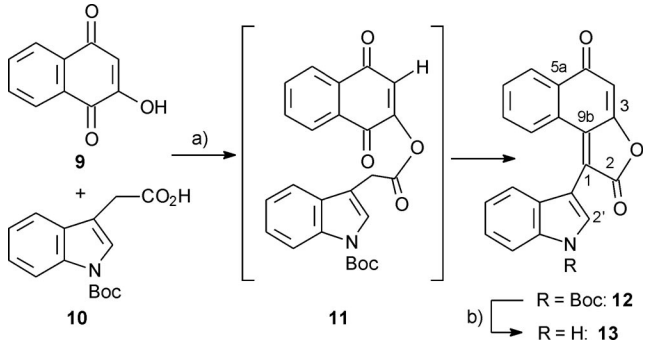
While the naphtoquinoid part of **1** and **2** originates from pentaketides derived from the 1,8-dihydroxynaphthalene melanin biosynthesis, the indole moiety of **1** and **2** apparently originates from tryptophan which is converted to 3-indolepyruvic acid by a transaminase. This proposal is supported by the fact, that **1** and **2** are only generated in significant quantities if tryptophan is present as sole nitrogen source in the medium. Moreover, we recently detected a transaminase in *Ustilago maydis*, which is responsible for the generation of indole alkaloids such as pityriacitrin in this species.[10] While 3-indolepyruvic acid is generated in an enzyme-controlled manner, no genes could be found which are responsible for the condensation of 3-indolepyruvic acid and tryptophan to pityriacitrin (**3**). Instead, we could demonstrate that traces of pityriactrin are generated spontaneously upon incubation of an aqueous solution of trypophan with 3-indolepyruvic acid.[10] We were also able to isolate pityriacitrin from *E. dermatitidis*. This compound has been found both in cultures of the wild-type strain and of the mutant Mel-1. Since *E. dermatitidis* and *U. maydis* are not closely related to each other, it is likely that **3** is also generated spontaneously in *E. dermatitidis* from 3-indolepyruvate and tryptophan, which are both ubiquitous primary metabolites.

The experience obtained during the investigation of the origin of pityriacitrin (**3**) led us to carry out some incubation experiments with potential precursors of exophialin. Incubation of both 2-hydroxyjuglone (**8**)[11] with 3-indoleacetic acid and of **8** with 3-indolepyruvic acid in aqueous solution, in methanol or in acetonitrile for 24 h at 25 °C led to the generation of a complex mixture of compounds, but

we were not able to detect exophialin (**2**) even by LC-HRMS (APCI). Neither could we detect any exophialin when we added silica gel or acetic acid to the various incubation mixtures. In contrast, **1** and **2** could be detected easily by LC-HRMS (APCI) directly from the crude extracts of the mutant Mel 1 and the wild-type strain of *E. dermatitidis*, respectively. Therefore, it can be excluded that exophialin is an artefact which has been generated during the work-up procedure.

Nevertheless, exophialin (**2**) is obviously not generated entirely by highly substrate-specific enzymes, since the mutant Mel-1 generates 8-hydroxyexophialin instead of exophialin. This means that either the condensation step is catalysed by a relatively unspecific enzyme, or the condensation occurs spontaneously without enzymatic support. However, it seems likely that additional enzymes – apart from the transaminase responsible for the generation of 3-indolepyruvic acid – are required for the production of exophialin (**2**) since the compound was not produced in the incubation experiments described above.

In order to evaluate the likelihood of a spontaneous, non-stereospecific condensation of 3-indoleacetic acid (**10**) with an aromatic pentaketide in more detail, we tried to synthesize the exophialin analogue 6-deoxyexophialin (**13**) biomimetically (Scheme 2). In accordance with the experiments to generate **2** from 3-indoleacetic acid and 2-hydroxyjuglone (**8**) all attempts failed to generate 6-deoxyexophialin (**13**) by direct condensation of 3-indoleacetic acid with 2-hydroxynaphthoquinone (**9**) under acidic, neutral and basic conditions. However, 6-deoxyexophialin (**13**) could be synthesized in relatively low yields after protecting the indole nitrogen of 3-indoleacetic acid with a *tert*-butyloxycarbonyl (Boc) group,[12] activation of the resulting carboxylic acid **10** with HATU and subsequent esterification with **9**.[13] The resulting ester intermediate **11** is obviously converted spontaneously to **12** in an intramolecular Knoevenagel condensation reaction (Scheme 2). Hence, it is likely that also exophialin **2** could be generated spontaneously from the corresponding ester **11**, thus explaining, why exophialin shows no axial chirality in nature.



Scheme 2. Synthesis of 6-deoxyexophialin. a) HATU, DIPEA, –78 °C, 15 min, 18%. b) Silica gel, 50 °C, 0.2 hPa, 12 h, 13%.

Moreover, the NMR spectroscopic data of 6-deoxyexophialin (**13**) are in good agreement with those of exophialin, thus confirming the structure proposed for exophialin (**2**). Significant differences occur only in the phenyl ring of the naphthoquinone moiety on account of the missing hydroxy group. Also, the UV/Vis spectrum of **13** resembles that of **2**.

Conclusions

Two new indole alkaloids, exophialin (**2**) and 8-hydroxyexophialin (**1**), have been isolated from cultures of the wild-type strain and the mutant Mel-1 of *E. dermatitidis*. The structures of these alkaloids are unusual since there are no other examples for indole alkaloids containing an aromatic pentaketide. However, a benzofurandione moiety is exhibited also in xylerithrin, deoxyxylerithrin and related natural products from the wood-rotting fungus *Peniophora sanguinea*.[14] Their biosynthesis seems to proceed via a condensation reaction of pulvinic acid with phenylpyruvate.[15]

Exophialin (**2**) and 8-hydroxyexophialin (**1**) are only produced in significant amounts if tryptophan is present as sole nitrogen source in the medium. Thus, the new natural products are generated by cultivation of the strains on a special medium, a method which has been applied before by Zeeck mostly to the cultivation of bacterial strains to tap the full potential of organisms in the production of secondary metabolites.[16]

Both exophialin (**2**) and 8-hydroxyexophialin (**1**) are apparently not completely synthesized under the control of highly substrate-specific enzymes, the last step in the biosynthesis might be a spontaneously occurring Knoevenagel condensation. Therefore, it was possible to elucidate the structure of exophialin (**2**) which is produced only in low yields from the wild-type strain by elucidating first the structure of 8-hydroxyexophialin (**1**) which is generated by the mutant Mel-1 in better yields. Also in the biosynthesis of some polyketides enzymes with low substrate specificity are involved. For instance, some of the late steps of the ansamitocin biosynthesis, an antibiotic produced by the actinomycete *Actinosynnema preciosum*, are interchangeable, thus leading to a metabolic grid.[17]

Moreover, it might even be advantageous for the fungus if bioactive compounds are not only generated by highly substrate-specific enzymes. A variety of bioactive indole alkaloids can be generated from 3-indolepyruvic acid in this manner.[10] For instance, it has been shown that pityriacitrin (**3**) is an efficient protector against UV light.[7] The pityriarubins[18] isolated from *Malassezia furfur* inhibit the oxidative burst of human granulocytes,[19] thus explaining why the skin is not inflamed on the parts which are infected by this fungus.

Experimental Section

General Experimental Procedures: Evaporation of the solvents was performed under reduced pressure using a rotary evaporator. Gel chromatography was performed with a Sephadex LH 20 column (50 × 3 cm, Pharmacia) and MeOH as eluent. For preparative thin layer chromatography the compound mixtures were applied with a

Linomat IV (Camag) onto silica gel 60 F_{254} precoated plates (20 × 20 cm × 1 mm, Merck). The plates were developed for 45 min with the solvent system toluene/HCO_2Et/HCO_2H (10:5:3, v/v/v). Preparative HPLC separations were performed on two different instruments. For the isolation of **1** and **2** a Gilson M 305 master pump, a Gilson 302 pump, a Gilson 802 gradient controller and a Gilson Holochrome UV detector was used (instrument 1). The samples were separated on a LiChrospher RP-8 column (10 μm, 30 × 250 mm, Merck). The purification of **12** and **13** was performed on a preparative HPLC consisting of two Waters 590EF pumps equipped with an automated gradient controller 680 and a Kratos Spectroflow 783 UV/Vis detector (instrument 2). The samples were separated on a Luna C-18 (2) column (5 μm, 15 × 250 mm, Phenomenex). UV/Vis spectra were recorded with a Varian Cary 100 Bio UV/Vis spectrometer. Optical rotation values were measured with a Jasco P-1030 polarimeter. CD spectra were obtained with a Jasco J-715 spectropolarimeter. NMR spectra were recorded with a Bruker DMX 600 spectrometer equipped with a TXI cryo probe (^1H at δ_H = 600.13, ^{13}C at δ_C = 150.9 MHz) and a Bruker DMX 500 spectrometer (^1H at δ_H = 500.11, ^{13}C at δ_C = 125.8 MHz). Chemical shifts were determined relative to the solvents CD_3OD (δ_H = 3.31, δ_C = 49.0 ppm) and $CDCl_3$ (δ_H = 7.26, δ_C = 77.0 ppm) as internal standards. MS (EI) spectra were obtained with a ThermoElectron DSQ instrument equipped with direct insertion probe using EI at 70 eV. HRMS (EI) spectra were obtained with a Finngian MAT 8200 instrument. LC-HRMS (APCI) and LC-HRMS-MS (APCI) spectra were obtained with an LTQ-Orbitrap Spectrometer (Thermo Scientific). The spectrometer was operated in positive or negative mode (1 spectrum s^{-1}; mass range: 50–1000) with nominal mass resolving power of 60 000 at *m/z* = 400 with a scan rate of 1 Hz with automatic gain control to provide high-accuracy mass measurements within 2 ppm deviation using polydimethylcyclosiloxane {$[(CH_3)_2SiO]_6$, *m/z* = 445.120025} as internal lock mass. The spectrometer was equipped with a Dionex HPLC system Ultimate 3000 consisting of pump, UV detector, flow manager and autosampler (injection volume 0.5 μL). Nitrogen was used as sheath gas (6 arbitrary units) and helium served as the collision gas. The HPLC was equipped with a Synergi Fusion column (4 μm, 3 × 150 mm, Phenomenex). The separations were performed with the following solvent mixtures and gradient programme: solvent A: 10 mmol NH_4OAc and 0.1% HCO_2H in H_2O; solvent B: 0.1% HCO_2H in MeCN; gradient: 3 min at 70% A, then linear in 25 min to 20% B; flow rate: 0.5 mL/min; UV detection, λ = 520 nm.

Fungal Strains and Culture Conditions: Strains: *E. dermatitidis* Mel-1: ATCC 44502 (American Type Culture Collection, Manassas, VA, USA), *E. dermatitidis*: CBS 207.35 (Centraalbureau voor Schimmelcultures, Utrecht, The Netherlands), ATCC 34100 (American Type Culture Collection, Manassas, VA, USA). Two hundred Petri dishes (90 mm diameter), each of them containing 10 g of solid pigment production inducing minimal medium, were inoculated with a suspension of *E. dermatitidis* by means of a swab and incubated for four weeks at 32 °C. The culture medium was prepared by autoclaving Tween® 80 (30 mL) and agar (20 g) dissolved in distilled water (1000 mL). After sterilisation and cooling to 50 °C, sterile filtered L-tryptophan (3.06 g, 15 mmol) and D-glucose (10 g) were added and the medium was poured in 10 mL portions on 100 plates.

Isolation of Indole Alkaloids: The cultures and the solid culture medium of 200 plates were homogenized and extracted for 12 h with ethyl acetate (3 L). The crude extract was filtered through a layer of glass wool and washed with water (3 L). Then, the organic phase was separated and dried with anhydrous sodium sulfate and the solvent was removed. The residue (3.1 g) was separated on a Sephadex LH 20 column with methanol as eluent. 8-Hydroxyexophialin (**1**) elutes within the fifth coloured band on Sephadex LH 20, exophialin (**2**) within the third coloured band and pityriacitrin (**3**) within the forth coloured band. Pigment containing fractions were each separated on 20 preparative silica gel 60 thin layer plates (20 × 20 cm, 1 mm layer thickness) with toluene/ethyl formate/formic acid (10:5:3, v/v/v) as eluent (**1**: R_f = 0.37; **2**: R_f = 0.54; **3**: R_f = 0.18). After drying, the respective red bands were scraped off and partitioned between H_2O and EtOAc. The EtOAc layer was separated and dried with anhydrous Na_2SO_4. After removal of sodium sulfate the solution was evaporated and the residue was dissolved in acetonitrile. Further purification of the fractions was achieved by preparative HPLC (instrument 1) on an RP-8 column (gradient programme: from 100% H_2O linear to 100% MeCN over 180 min; flow rate: 5 mL/min; detection: UV at 220 nm). The eluting compounds were collected in 5 mL fractions with a fraction collector. Fractions containing the same compound were combined and freeze-dried (**1**: R_t = 129–140 min, yield 1.78 mg; **2**: R_t = 129–140 min, yield 0.43 mg; **3**: R_t = 120–130 min, yield 0.56 mg).

8-Hydroxyexophialin (1): Red solid; m.p. 178 °C (dec.). $[\alpha]_D^{25}$ (MeOH): no optical rotation. CD (MeOH): no Cotton effects. ^1H NMR: see Table 1. ^{13}C NMR: see Table 1. UV/Vis (MeOH): λ_{max} (lgε) = 218 (4.29), 274 (3.66), 318 (3.63), 501 (3.75) nm. MS (– APCI): *m/z* = 344. MS-MS (–APCI, parent ion *m/z* = 344, 40 eV): *m/z* (%) = 344 (62) $[M - H]^-$, 328 (93) $[M - H + H_2 - H_2O]^-$, 317 (35) $[M - H - HCN]^-$, 316 (17) $[M - H - CO]^-$, 299 (4) $[M - H - HCN - H_2O]^-$, 287 (4), 275 (26), 273 (77) $[M - H - HCN - H_2O - C_2H_2]^-$, 271 (100) $[M - H - HCN - H_2O - CO]^-$, 247 (12) $[M - H - HCN - H_2O - C_2H_2 - C_2H_2]^-$, 245 (32) $[M - H - HCN - H_2O - CO - C_2H_2]^-$, 243 (40) $[M - H - HCN - H_2O - CO - CO]^-$, 231 (9), 217 (15) $[M - H - HCN - H_2O - CO - C_2H_2 - CO]^-$. LC-HRMS (APCI): R_t = 12.2 min, calcd. for $C_{20}H_{12}NO_5$ $[M + H]^+$ 346.0710; found 346.0709.

Exophialin (2): Red solid; m.p. 168 °C (dec.). $[\alpha]_D^{25}$ (MeOH): no optical rotation. CD (MeOH): no Cotton effects. ^1H NMR: see Table 1. ^{13}C NMR: see Table 1. UV/Vis (MeOH): λ_{max} (lgε) = 204 (4.30), 270 (3.67), 308 (3.64), 525 (3.55) nm. HRMS (–APCI): *m/z* = 328.0612 $[M - H]^-$ {calcd. for $C_{20}H_{10}NO_4$ $[M - H]^-$ 328.0615; found 328.0612}. HRMS/MS: (–APCI, parent ion *m/z* = 328, 35 eV): *m/z* (%) = 328.0616 (5) $[C_{20}H_{10}NO_4]^-$, 300.0654 (100) $[C_{19}H_{10}NO_3]^-$; LC-HRMS (APCI): R_t = 15.0 min, calcd. for $C_{20}H_{12}NO_4^+$ $[M + H]^+$ 330.0761; found 330.0761.

Detection of 1 and 2 in the Crude Extract of *E. dermatitidis* (CBS 207.35): The solid medium on which *E. dermatitidis* had been grown was broken up into small pieces and extracted twice with 100 mL of ethyl acetate for 12 h. The extract was filtered and the solvent was removed. Then, the solid residue was dissolved in a mixture consisting of H_2O and MeCN (1:1, v/v) and analysed by LC-HRMS (APCI) (see also General Experimental Procedures) **1**: R_t = 12.2 min; **2**: R_t = 15.0 min.

Experiments on the Generation of Exophialin (2) from Potential Precursors: 3-Indolepyruvic acid (1.06 mg, 5.26 μmol) and 2,5-dihydroxy-1,4-naphthoquinone (2-hydroxyjuglone, **8**, 1.00 mg, 5.26 mol) were stirred for 24 h at 25 °C with different solvents and additives. The same experiments were repeated with 3-indoleacetic acid (0.92 mg, 5.26 μmol) and 2,5-dihydroxy-1,4-naphthoquinone (2-hydroxyjuglone (**8**), 1.00 mg, 5.26 μmol).[10] As solvents H_2O (1 mL), MeOH (1 mL) or MeCN (1 mL) were used. The experiments were also performed in H_2O (1 mL) containing AcOH (0.1 mL), in MeOH (1 mL) containing AcOH (0.1 mL) and in MeCN (1 mL) containing AcOH (0.1 mL). In addition, the experiments were repeated in H_2O (1 mL) containing silica gel (30 mg), in MeOH

(1 mL) containing silica gel (30 mg) and in MeCN (1 mL) containing silica gel (30 mg). Solids were filtered off after 24 h and solvents were removed from each sample. The residues (18 different samples) were dissolved separately in a mixture of H_2O and MeCN (1:1, v/v) and analysed by LC-HRMS (APCI) using the same conditions as for the detection of **2** from the crude extract. An ion trace search for m/z = 330.0761, the $[M + H]^+$ ion of **2**, led to no hints for the presence of this ion in all samples.

***N*-(*tert*-Butyloxycarboxyl)-6-deoxyexophialin (12):** A solution of 2-[1-(*tert*-butyloxycarbonyl)-1*H*-indol-3-yl]acetic acid (200 mg, 0.727 mmol) in absolute DMF (4 mL) was cooled under an argon atmosphere to –78 °C and DIPEA (diisopropylethylamine, 0.140 mL, 0.799 mmol) and HATU[12] [*N*,*N*,*N*′,*N*′-tetramethyl-*O*-(7-azabenzotriazol-1-yl)uronium hexafluorophosphate, 276 mg, 0.727 mmol] were added. After 15 min 2-hydroxynaphthoquinone (506 mg, 2.91 mmol) was added to the stirred solution. Then, the reaction mixture was warmed up to room temperature. After 12 h, the solvent was removed in vacuo. The residue was dissolved in ethyl acetate (100 mL). The resulting solution was washed twice with water (2 × 25 mL) and once with a solution of saturated NaCl (25 mL). The organic layer was dried with anhydrous sodium sulfate, the solvent was removed in vacuo and the residue was purified by flash chromatography on silica gel with hexane/ethyl acetate (20:1). The solvents were removed in vacuo and the residue was purified by preparative HPLC (instrument 2) on an RP-18 column (gradient: 5 min at 69.97% H_2O, 0.03% HOAc, 30.0% MeCN, then linear to 100% MeCN over 30 min, flow rate 12 mL min^{-1}; detection: UV at 430 nm; R_t = 29.2 min); yield 53 mg (18%), yellow solid, m.p. 137 °C (dec.). R_f (silica gel, solvent: hexane/ethyl acetate, 20:1): 0.09. ^1H NMR (500 MHz, $CDCl_3$, 300 K): δ = 1.73 [s, 9 H, $(CH_3)_3$], 6.40 (s, 1 H, 4-H), 7.09 (dd, J = 7.9, 1.0 Hz, 1 H, 4′-H), 7.21 (ddd, J = 7.9, 7.2, 0.8 Hz, 1 H, 5′-H), 7.36 (ddd, J = 7.9, 7.4, 1.0 Hz, 1 H, 8-H), 7.43 (ddd, $^3J_{HH}$ = 8.4, 7.2, 1.0 Hz, 1 H, 6′-H), 7.51 (dd, J = 7.9, 1.0 Hz, 1 H, 9-H), 7.58 (ddd, J = 7.8, 7.4, 1.0 Hz, 7-H), 8.16 (s, 1 H, 2′-H), 8.28 (dd, J = 7.8, 1.0 Hz, 1 H, 6-H), 8.32 (dd, J = 8.4, 0.8 Hz, 1 H, 7′-H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$, 300 K): δ = 28.2 [$(CH_3)_3$], 85.3 [$C(CH_3)_3$], 106.4 (C-4), 109.1 (C-3′), 116.0 (C-7′), 121.5 (C-4′), 121.8 (C-1), 123.3 (C-5′), 125.6 (C-6′), 126.7 (C-3a′), 127.6 (C-9a), 127.7 (C-6), 128.5 (C-9), 129.4 (C-2′), 131.1 (C-5a), 131.6 (C-7), 132.4 (C-8), 135.6 (C-7a′), 137.3 (C-9b), 148.9 [O(*C*O)N], 161.4 (C-3a), 167.6 (C-2), 184.4 (C-5) ppm. UV/Vis (MeCN): λ_{max} (lg ε) = 224 (2.69), 256 (2.35), 303 (2.52), 430 (2.23) nm. MS (EI): m/z (%) = 413 (5) $[M]^+$, 357 (43), 313 (100), 285 (19), 256 (8), 228 (47), 200 (12), 114 (10), 100 (9), 57 (77), 44 (30). HRMS (EI): calcd. for $C_{25}H_{19}NO_5$ $[M]^+$ 413.1263; found 413.1258.

6-Deoxyexophialin (13): *N*-(*tert*-Butyloxycarboxyl)-6-deoxyexophialin (**12**, 20 mg, 0.048 mmol) was dissolved in CH_2Cl_2 (10 mL) and mixed with silica gel (500 mg). Then, the solvent was removed in vacuo. The residue was warmed up to 50 °C at 0.2 hPa for 12 h.[20] Remaining starting material was removed by washing the silica gel with CH_2Cl_2. (The latter can be used once more for deprotection.) Then the silica gel was extracted with MeOH, the extract was filtered, the solvent removed in vacuo and the residue purified by preparative HPLC (instrument 2) on an RP-18 column (gradient: 5 min at 49.95% H_2O, 0.05% HOAc, 50% MeOH, then linear to 100% MeOH over 30 min; flow rate: 6 mL min^{-1}; detection: UV at 500 nm; R_t = 28.2 min); yield 2 mg (13%), red solid, m.p. 157 °C. R_f (silica gel, hexane/ethyl acetate, 4:1): 0.16. ^1H NMR (600 MHz, CD_3OD, 280 K): δ = 6.35 (s, 1 H, 4-H), 6.99 (d, J = 8.0 Hz, 1 H, 4′-H), 7.11 (dd, J = 8.0, 6.9 Hz, 1 H, 5′-H), 7.29 (dd, J = 8.1, 6.9 Hz, 1 H, 6′-H), 7.46 (dd, J = 8.0, 6.8 Hz, 1 H, 8-H), 7.586 (d, J = 8.0 Hz, 1 H, 9-H), 7.592 (d, J = 8.1 Hz, 1 H, 7′-H), 7.606 (dd, J = 7.8, 6.8 Hz, 1 H, 7-H), 8.07 (s, 1 H, 2′-H), 8.24 (d, J = 7.8 Hz, 1 H, 6-H) ppm. ^1H NMR (600 MHz, CD_3OD, 300 K): δ = 6.34 (s, 1 H, 4-H), 7.00 (d, J = 7.7 Hz, 1 H, 0.8 Hz, 4′-H), 7.10 (ddd, J = 7.7, 7.4, 0.7 Hz, 1 H, 5′-H), 7.29 (ddd, J = 7.9, 7.4, 0.8 Hz, 1 H, 6′-H), 7.45 (ddd, J = 8.1, 7.2, 1.3 Hz, 1 H, 8-H), 7.58–7.61 (m, 3 H, 7-H/7′-H/9-H), 8.05 (s, 1 H, 2′-H), 8.24 (dd, J = 8.1, 1.3 Hz, 1 H, 6-H) ppm. ^{13}C NMR (151 MHz, CD_3OD, 300 K): δ = 105.4 (C-4), 106.4 (C-3′), 113.8 (C-7′), 121.9 (C-5′), 123.1 (C-4′), 124.1 (C-6′), 125.8 (C-3a′), 128.1 (C-6), 129.5 (C-9), 130.0 (C-9a), 131.5 (C-7), 132.2 (C-5a), 132.5 (C-2′), 133.4 (C-8), 138.5 (C-7a′), 164.5 (C-3a), 186.7 (C-5) ppm. UV/Vis (MeOH): λ_{max} (lg ε) = 202 (2.90), 306 (2.33), 492 (2.31) nm. MS (EI): m/z (%) = 313 (100) $[M]^+$, 285 (14), 257 (12), 228 (73), 200 (12), 129 (6), 115 (11). HRMS (EI): calcd. for $C_{20}H_{11}NO_3$ $[M]^+$ 313.0739; found 313.0735.

Supporting Information (see also the footnote on the first page of this article): Selected UV/Vis, NMR and mass spectra of compounds **1**, **2**, **3** and **13**.

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