A Novel Alkaloid Serantrypinone and the Spiro Azaphilone Daldinin D from *Penicillium thymicola*

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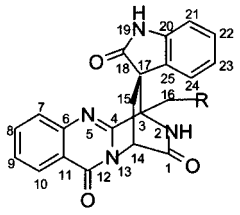
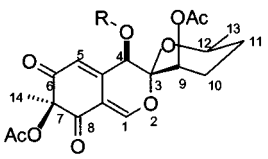
The novel quinazoline metabolite serantrypinone (**1**) has been isolated from an isolate of the microfungus *Penicillium thymicola* together with daldinin D (**2**), a new peracetylated spiro azaphilone derivative. The structures of **1** and **2** were elucidated by analysis of spectroscopic data, including 2D NMR, and comparison with literature data.

Recently a large number of suspected ochratoxin A-producing *Penicillium* isolates were investigated in a chemotaxonomic study.[1] The study divided the isolates into two large groups representing the two species *P. verrucosum* and *P. nordicum* together with a small group of four non-ochratoxin-producing isolates. This latter group included the isolate of *P. thymicola* recently studied by our group.[2]

In addition to displaying different morphological characteristics the three species can be distinguished by differences in production of quinazoline and other secondary metabolites.[1] *P. verrucosum* produces verrucines,[3] *P. nordicum* produces anacines,[1] while *P. thymicola* produces anacine, fumiquinazoline F, and the related spiroquinazoline alantrypinone.[1,2] From previous work with UV-guided isolation of secondary metabolites from *P. thymicola*[2] we knew that the fungus often produces a minor compound with UV data almost identical to those seen for a major component, alantrypinone (**4**).[2] The present report describes the isolation and structure elucidation of this minor and novel quinazoline compound, serantrypinone (**1**), together with a new azaphilone derivative, daldinin D (**2**), from *P. thymicola* (IBT 5891).



1 R = OH
4 R = H



2 R = CH_3CO
3 R = $CH_3CH{=}C(CH_3)CO$

The molecular composition of serantrypinone ($C_{21}H_{16}O_4N_4$) was established by HREIMS ([M^+] at *m*/*z* 388.1170). This result is consistent with the presence of an additional oxygen atom in **1** when compared to **4**. NMR data of **1** showed signals characteristic of an oxindole unit as in **4** and a quinazoline moiety derived from anthranilic acid. HMBC experiments predicted the linkage between C-3 in the oxopiperazine ring and C-17 in the tryptophane-derived oxindole as in **4**. However, a significant difference between the two compounds was revealed by the chemical shift value of C-16, indicating the oxygenation in **1**. In agreement with this assignment, H-16 appeared as two doublets at δ 3.57 and 3.71 instead of the singlet at δ 1.19 in **4**. On the basis of this information, **1** can be established as a new structure resulting from the exchange of the alanine residue in **4** for a serine residue. The compound is named serantrypinone, according to the nomenclature of Penn et al.[4] The circular dichroism spectrum of **1** was similar to that of alantrypinone,[2] enabling the assignment of the corresponding absolute configuration at C-3 (3*R*), C-14 (14*R*), and C-17 (17*S*).[3]

A new azaphilone derivative, daldinin D (**2**), was also isolated from IBT 5891. The molecular formula of daldinin D ($C_{21}H_{24}O_{10}$) was established by HREIMS ([M^+] at *m*/*z* 436.1369). The ^{1}H and ^{13}C NMR data showed three acetate groups and two trisubstituted olefinic units. The UV spectrum displayed absorptions characteristic of $\alpha,\beta,\gamma,\delta$-conjugated carbonyl groups. These data, together with careful investigation of the H−H COSY and HMBC spectra, led to the establishment of the tricyclic spiro-acetal structure **2** almost identical to that of daldinin C produced by *Daldinia concentrica*.[5] Compounds **2** and **3** only differ with respect to their substitution at position 4. The linkage of the partial structures was inferred from the long-range correlations observed between H-1, H-4, H-9 and CH_3-13 to C-3, a quaternary carbon at δ 100.9. This ^{13}C NMR chemical shift is characteristic of acetal groups. The absolute configuration of **3** was established by X-ray crystallography and chemical degradation.[5] The CD spectrum of **2** was similar to that of an authentic sample of **3** (see experimental data for both compounds), suggesting identical absolute configuration. The stereostructure proposed is coherent with the correlations observed in the NOESY spectrum of **2** (Figure 1).

Interestingly, the likely polyketide-derived structure of **2** resembles those of ochratoxin A produced by the closely related species *P. verrucosum* and *P. nordicum*, and citrinin produced only by *P. verrucosum*. This indicates that the three species recently demonstrated[1] to share the capability to produce the same pattern of volatile metabolites and

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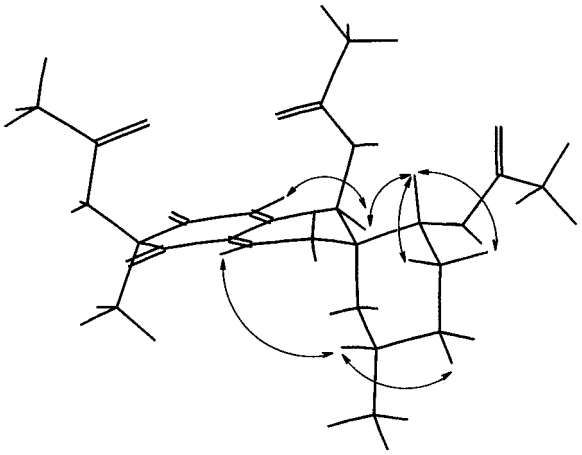

Figure 1. Important NOE correlations observed in daldinin D (**2**).

verrucolone metabolites might also have very similar genes for polyketide biosynthesis.

In conclusion, the present study describes some further metabolites unique to *P. thymicola*. Detection of these compounds in either pure fungal cultures or in contaminated food and feed stuffs strongly indicates the producing microorganism to be *P. thymicola* and not one of the closely related ochratoxin A-producing species *P. verrucosum* and *P. nordicum*.

Experimental Section

General Experimental Procedures. NMR spectra of daldinin D (**2**) were recorded in $CDCl_3$ on a Varian 400 FT-NMR spectrometer at 400.0 and 100.6 MHz for ^{13}C and ^{1}H NMR spectra, respectively. NMR spectra of serantrypinone (**1**) were recorded in 5 mm tubes at 600.13 MHz for ^{1}H and at 150.92 MHz for ^{13}C and at 300 K, using DMSO-d_6, on a Bruker DRX 600 according to Larsen et al.[6] The chemical shifts are given relative to DMSO, 2.50 ppm for H^1 and 39.5 ppm for ^{13}C. EIMS originate from a JEOL JMS-HX/HX110A tandem mass spectrometer. The circular dichroism (CD) spectra were measured on a JASCO J-710 spectropolarimeter and the UV spectra on a Hewlett-Packard 8452A diode array spectrophotometer. Analytical HPLC conditions were similar to those given by Smedsgaard,[7] and retention indices (RI) of fungal metabolites were calculated according to Frisvad and Thrane.[8]

The 3D structure of daldinin D (Figure 1) was drawn using CS Chem 3D Pro, Molecular Modeling and Analysis, Version 3.2, CambridgeSoft Corporation, MA.

Fungal Material and Fermentation. The *Penicillium* isolate (IBT 5891) was obtained from the IBT Culture Collection at BioCentrum-DTU, Technical University of Denmark. The fungus was cultured for 14 days in 10 conical flasks (1 L), each containing 200 mL of SYES liquid medium according to Svendsen and Frisvad,[9] however, without agar.

Extraction and Separation. The combined fungal mycelia were extracted twice for 16 h at room temperature with 300 mL of EtOAc and filtered through a Whatman 1PS phase separation filter before evaporation to give approximately 3.5 g of crude extract. This extract was subjected to vacuum-liquid chromatography on silica gel[10] to give a fraction rich in serantrypinone (**1**) and one rich in daldinin D (**2**). The fraction (420 mg) rich in **1** was further purified on a Merck Lichroprep RP-18 (25 × 310 mm, 40−63 μm) column (H_2O−MeOH, 50:50, 20 mL/min), giving five fractions. The first fraction (42 mg) was purified on a Waters Prep Nova-Pak Porasil cartridge (8 × 100 mm, 6 μm, 60 Å) using 2 mL/min H_2O−MeOH (70:30 to 30:70 in 20 min) as mobile phase to give 6 mg of pure **1**. The fraction (520 mg) rich in **2** was purified on a Waters Prep Nova-Pak Porasil cartridge (25 × 100 mm, 6 μm, 60 Å) using 20 mL/min H_2O−CH_3CN (50:50) as mobile phase to give 41 mg of pure **2**.

Serantrypinone (1): $[\alpha]^{22}_D$ −12° (*c* 0.12, EtOH); UV λ_{max} (EtOH) nm (log ϵ) 218 (4.55), 267 (3.54), 278 (3.51), 290 (3.36) 305 (3.16), 318 (3.05); CD (EtOH, *c* 0.08), $\Delta\epsilon$ (λ nm) 235 (−1.54), 246 (+0.91), 274 (−0.97), 295 (+0.35), 318 (−0.14); ^{1}H NMR δ 8.22 (1H, br d, J = 8.0 Hz, H-10), 7.89 (1H, br t, J = 7.5 Hz, H-8), 7.75 (1H, br d, J = 8.0 Hz, H-7), 7.61 (1H, br t, J = 7.5 Hz, H-9), 7.29 (1H, br t, J = 7.5 Hz, H-22), 7.22 (1H, br d, J = 7.5 Hz, H-24), 7.07 (1H, br t, J = 7.5 Hz, H-23), 6.89 (1H, br d, J = 8.0 Hz, H-21), 5.55 (1H, dd, J = 3.4, 2.0 Hz, H-14), 3.71 (1H, d, J = 11.7 Hz, H-16a), 3.57 (1H, d, J = 11.7 Hz, H-16b), 2.41 (1H, dd, J = 14.3, 3.4 Hz, H-15a), 2.36 (1H, dd, J = 14.3, 2.0 Hz, H-15b); ^{13}C NMR δ 176.8 (C-18), 169.6 (C-1), 158.3 (C-12), 152.0 (C-4), 146.5 (C-6), 142.4 (C-20), 134.5 (C-8), 129.6 (C-25), 128.9 (C-22), 127.6 (C-7), 127.1 (C-9), 126.1 (C-10), 123.6 (C-24), 121.8 (C-23), 120.1 (C-11), 109.6 (C-21), 64.9 (C-3), 57.6 (C-16), 52.5 (C-17), 51.9 (C-14), 37.3 (C-15). The following HMBC correlations were observed: from C-1 to H-14 and H-15; from C-3 to H-16; from C-4 to H-14 and H-16; from C-6 to H-8 and H-10; from C-7 to H-9; from C-8 to H-10; from C-9 to H-7; from C-10 to H-8; from C-11 to H-7 and H-9; from C-12 to H-10; from C-17 to H-14, H-15, H-16, and H-24; from C-18 to H-15; from C-20 to H-22 and H-24; from C-21 to H-23; from C-22 to H-24; from C-23 to H-21; from C-24 to H-22; and from C-25 to H-15, H-21, and H-23; HREIMS *m/z* 388.1172 (−0.3 mmu calcd for $C_{21}H_{16}O_4N_4$); RI = 637.[8]

Daldinin D (2): mp 154−156 °C; $[\alpha]^{22}_D$ −19° (*c* 0.01, EtOH); UV λ_{max} (EtOH) nm (log ϵ) 305 (4.01), 375 sh (3.13), 455 (3.19); CD (EtOH, *c* 0.04), $\Delta\epsilon$ (λ nm) 218 (−9.95), 302 (+8.09), 346 (−1.99), 379 (+1.62); ^{1}H NMR δ 7.62 (1H, d, J = 1.5 Hz, H-1), 6.13 (1H, d, J = 1.5 Hz, H-5), 5.74 (1H, s, H-4), 5.13 (1H, br t, J = 2.8 Hz, H-9), 3.83 (1H, ddq, J = 11.0, 2.7, 6.4 Hz, H-12), 2.14 (3H, s, CH_3CO$_2$-7), 2.12 (1H, m, H-10), 2.08 (3H, s, CH_3CO$_2$-9), 1.99 (3H, s, CH_3CO$_2$-4), 1.90 (1H, m, H-10), 1.65 (1H, m, H-11), 1.55 (1H, m, H-11), 1.50 (1H, s, H-14), 1.12 (1H, d, J = 6.4 Hz, H-13); ^{13}C NMR δ 193.8 (C-6), 191.8 (C-8), 169.6 (CH$_3$*C*O$_2$-9), 169.5 (CH$_3$*C*O$_2$-4), 169.0 (CH$_3$*C*O$_2$-7), 153.9 (C-1), 141.5 (C-4a), 122.1 (C-5), 110.6 (C-8a), 100.9 (C-3), 84.8 (C-7), 69.0 (C-12), 65.3 (C-4), 63.4 (C-9), 26.2 (C-11), 23.4 (C-10), 22.1 (C-14), 21.1 (C-13), 20.8 (*C*H$_3$CO$_2$-9), 20.6 (*C*H$_3$CO$_2$-4), 19.9 (*C*H$_3$CO$_2$-7). The following HMBC correlations were observed: from C-3 to H-1, H-4, H-9, and 13-CH_3; from C-4 to CH_3CO$_2$-4 and H-5; from CH$_3$*C*O$_2$-4 to H-4 and CH_3CO$_2$-4; from C-4a to H-1 and H-4; from C-5 to H-4; from C-6 to CH_3-14; from C-7 to H-5 and CH_3CO$_2$-7, CH$_3$-14; from CH$_3$*C*O$_2$-7 to CH_3CO$_2$-7; from C-8 to H-1, H-4, and CH_3-14; from C-8a to H-1, H-4, and H-5; from C-9 to CH_3CO$_2$-9; from CH$_3$*C*O$_2$-9 to H-9 and CH_3CO$_2$-9; from C-11 to H-9 and CH_3-13; from C-12 to CH_3-13; from C-13 to CH_2-11; HREIMS *m/z* 436.1369 (−0.4 mmu calcd for $C_{21}H_{24}O_{10}$); RI = 1016.[8]

Daldinin C **(3):** $[\alpha]^{22}_D$ −34° (*c* 0.01, EtOH); CD (EtOH, *c* 0.04), $\Delta\epsilon$ (λ nm) 238 (−9.79), 308 (+10.74), 350 (−1.25), 387 (+0.55).

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