

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

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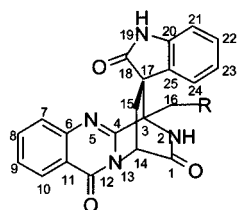
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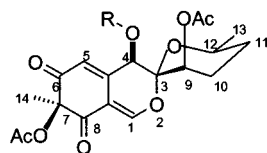
The novel quinazoline metabolite serantrypinone (**1**) has been isolated from an isolate of the microfungus *Penicillium thymicola* together with daldinin D (**2**), a new peracetylated spiro azaphilone derivative. The structures of **1** and **2** were elucidated by analysis of spectroscopic data,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

In addition to displaying different morphological characteristics the three species can be distinguished by differences in production of quinazoline and other secondary metabolites.<sup>1</sup> *P. verrucosum* produces verrucines,<sup>3</sup> *P. nordicum* produces anacines,<sup>1</sup> while *P. thymicola* produces anacine, fumiquinazoline F, and the related spiroquinazoline alantrypinone.<sup>1,2</sup> From previous work with UV-guided isolation of secondary metabolites from *P. thymicola*<sup>2</sup> we knew that the fungus often produces a minor compound with UV data almost identical to those seen for a major component, alantrypinone (**4**).<sup>2</sup> 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<sub>3</sub>CO  
**3** R = CH<sub>3</sub>CH=C(CH<sub>3</sub>)CO

The molecular composition of serantrypinone (C<sub>21</sub>H<sub>16</sub>O<sub>4</sub>N<sub>4</sub>) was established by HREIMS ([M<sup>+</sup>] 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  $\delta$  3.57 and 3.71 instead of the singlet at  $\delta$  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.<sup>4</sup> The circular dichroism spectrum of **1** was similar to that of alantrypinone,<sup>2</sup> enabling the assignment of the corresponding absolute configuration at C-3 (3*R*), C-14 (14*R*), and C-17 (17*S*).<sup>3</sup>

A new azaphilone derivative, daldinin D (**2**), was also isolated from IBT 5891. The molecular formula of daldinin D (C<sub>21</sub>H<sub>24</sub>O<sub>10</sub>) was established by HREIMS ([M<sup>+</sup>] at *m/z* 436.1369). The <sup>1</sup>H and <sup>13</sup>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*.<sup>5</sup> 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<sub>3</sub>-13 to C-3, a quaternary carbon at  $\delta$  100.9. This <sup>13</sup>C NMR chemical shift is characteristic of acetal groups. The absolute configuration of **3** was established by X-ray crystallography and chemical degradation.<sup>5</sup> 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<sup>1</sup> to share the capability to produce the same pattern of volatile metabolites and

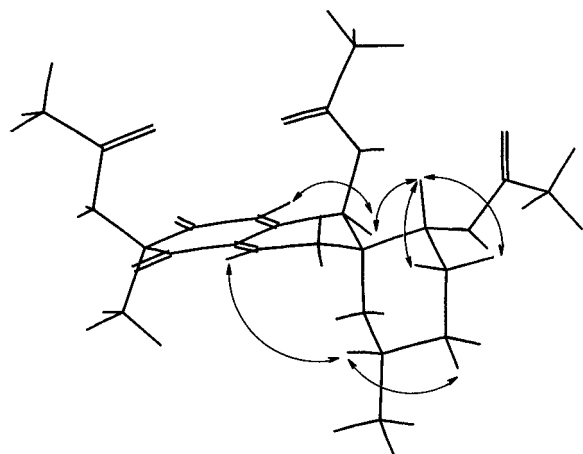
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**Figure 1.** Important NOE correlations observed in daldinin D (**2**).

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  $\text{CDCl}_3$  on a Varian 400 FT-NMR spectrometer at 400.0 and 100.6 MHz for  $^{13}\text{C}$  and  $^1\text{H}$  NMR spectra, respectively. NMR spectra of serantrypinone (**1**) were recorded in 5 mm tubes at 600.13 MHz for  $^1\text{H}$  and at 150.92 MHz for  $^{13}\text{C}$  and at 300 K, using  $\text{DMSO}-d_6$ , on a Bruker DRX 600 according to Larsen et al.<sup>6</sup> The chemical shifts are given relative to DMSO, 2.50 ppm for  $\text{H}^1$  and 39.5 ppm for  $^{13}\text{C}$ . 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,<sup>7</sup> and retention indices (RI) of fungal metabolites were calculated according to Frisvad and Thrane.<sup>8</sup>

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,<sup>9</sup> 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<sup>10</sup> 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  $\mu\text{m}$ ) column ( $\text{H}_2\text{O}$ –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  $\mu\text{m}$ , 60 Å) using 2 mL/min  $\text{H}_2\text{O}$ –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  $\mu\text{m}$ , 60 Å) using 20 mL/min  $\text{H}_2\text{O}$ – $\text{CH}_3\text{CN}$  (50:50) as mobile phase to give 41 mg of pure **2**.

**Serantrypinone (1):**  $[\alpha]_D^{22} -12^\circ$  ( $c$  0.12, EtOH); UV  $\lambda_{\text{max}}$  (EtOH) nm (log  $\epsilon$ ) 218 (4.55), 267 (3.54), 278 (3.51), 290 (3.36), 305 (3.16), 318 (3.05); CD (EtOH,  $c$  0.08),  $\Delta\epsilon$  ( $\lambda$  nm) 235 (–1.54), 246 (+0.91), 274 (–0.97), 295 (+0.35), 318 (–0.14);  $^1\text{H}$  NMR  $\delta$  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);  $^{13}\text{C}$  NMR  $\delta$  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  $\text{C}_{21}\text{H}_{16}\text{O}_4\text{N}_4$ ); RI = 637.<sup>8</sup>

**Daldinin D (2):** mp 154–156 °C;  $[\alpha]_D^{22} -19^\circ$  ( $c$  0.01, EtOH); UV  $\lambda_{\text{max}}$  (EtOH) nm (log  $\epsilon$ ) 305 (4.01), 375 sh (3.13), 455 (3.19); CD (EtOH,  $c$  0.04),  $\Delta\epsilon$  ( $\lambda$  nm) 218 (–9.95), 302 (+8.09), 346 (–1.99), 379 (+1.62);  $^1\text{H}$  NMR  $\delta$  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,  $\text{CH}_3\text{CO}_2-7$ ), 2.12 (1H, m, H-10), 2.08 (3H, s,  $\text{CH}_3\text{CO}_2-9$ ), 1.99 (3H, s,  $\text{CH}_3\text{CO}_2-4$ ), 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);  $^{13}\text{C}$  NMR  $\delta$  193.8 (C-6), 191.8 (C-8), 169.6 ( $\text{CH}_3\text{CO}_2-9$ ), 169.5 ( $\text{CH}_3\text{CO}_2-4$ ), 169.0 ( $\text{CH}_3\text{CO}_2-7$ ), 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 ( $\text{CH}_3\text{CO}_2-9$ ), 20.6 ( $\text{CH}_3\text{CO}_2-4$ ), 19.9 ( $\text{CH}_3\text{CO}_2-7$ ). The following HMBC correlations were observed: from C-3 to H-1, H-4, H-9, and 13- $\text{CH}_3$ ; from C-4 to  $\text{CH}_3\text{CO}_2-4$  and H-5; from  $\text{CH}_3\text{CO}_2-4$  to H-4 and  $\text{CH}_3\text{CO}_2-4$ ; from C-4a to H-1 and H-4; from C-5 to H-4; from C-6 to  $\text{CH}_3-14$ ; from C-7 to H-5 and  $\text{CH}_3\text{CO}_2-7$ ,  $\text{CH}_3-14$ ; from  $\text{CH}_3\text{CO}_2-7$  to  $\text{CH}_3\text{CO}_2-7$ ; from C-8 to H-1, H-4, and  $\text{CH}_3-14$ ; from C-8a to H-1, H-4, and H-5; from C-9 to  $\text{CH}_3\text{CO}_2-9$ ; from  $\text{CH}_3\text{CO}_2-9$  to H-9 and  $\text{CH}_3\text{CO}_2-9$ ; from C-11 to H-9 and  $\text{CH}_3-13$ ; from C-12 to  $\text{CH}_3-13$ ; from C-13 to  $\text{CH}_2-11$ ; HREIMS  $m/z$  436.1369 (–0.4 mmu calcd for  $\text{C}_{21}\text{H}_{24}\text{O}_{10}$ ); RI = 1016.<sup>8</sup>

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

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