UV-Guided Isolation of Alantrypinone, a Novel *Penicillium* Alkaloid

Thomas Ostenfeld Larsen,*,† Karla Frydenvang,‡ Jens Christian Frisvad,† and Carsten Christophersen§

Mycology Group, Department of Biotechnology, Technical University of Denmark, DK-2800 Lyngby, Denmark, Department of Medicinal Chemistry, Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark, and Marine Chemistry Section, The H.C.Ø. Institute, University of Copenhagen, DK-2100 Copenhagen, Denmark

Received February 19, 1998

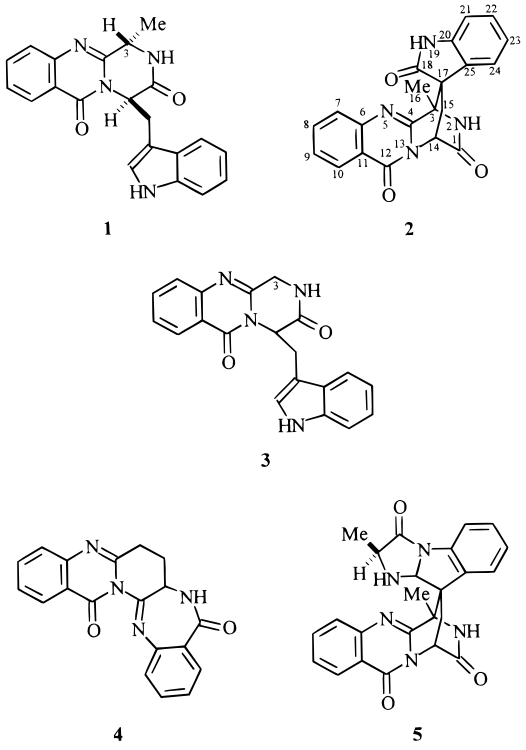
Fumiquinazoline F (**1**) and alantrypinone (**2**) have been isolated as the two major metabolites of *Penicillium thymicola*. The structure of **2**, which contains a new ring structure, was elucidated by analysis of spectroscopic data including 2D NMR. The absolute configuration of **2** was established by a single-crystal X-ray diffraction study.

During studies of cheese-associated *Penicillium verrucosum*, characterized by arabenoic acid[1] and ochratoxin A,[2] isolates with secondary-metabolite profiles different from typical *P. verrucosum* were detected. HPLC analysis with diode array detection of an extract of the new species *Penicillium thymicola*, to be described,[3] revealed similarity between the UV spectra of two major metabolites and those of glyantrypine (**3**)[4] and auranthine (**4**).[5] Retention indices, however, indicated the presence of two new *Penicillium* metabolites.

The present study reports the identification of the two compounds as fumiquinazoline F (**1**), previously characterized from *Aspergillus fumigatus*,[6] and a novel alkaloid, alantrypinone (**2**), named according to the nomenclature of Penn et al.[4] The carbon skeleton of **2**, however, is a new ring system very similar to the skeleton of the spiroquinazoline (**5**) from *A. flavipes*.[7]

MS data of **1** indicated a molecular weight of 358 amu, 14 amu higher than that of **3,** consistent with an additional methyl group in **1**. ^{1}H NMR of **1** established a methyl group at position 3, and comparison with literature data revealed **1** to be identical to fumiquinazoline F.[6]

The ^{13}C NMR of **2** showed 21 carbons, as in **1**, strongly indicating homogeneity. Despite being recorded in different solvents, C-4 to C-12 proved to be almost identical in carbon chemical shift values when compared to the data of **1**,[6] establishing a quinazoline moiety in **2**. The H−H COSY spectrum of **2** showed the presence of two 1,2-disubstituted benzene ring systems and a −CH−CH$_2$− sequence, indicating a tryptophan ring as in **1**. Changes in ^{13}C chemical shift values could be observed for C-17 and C-18, however, which could be assigned as a quaternary aliphatic carbon at 54.8 ppm and a carbonyl group at 176.6 ppm. Likewise, smaller deviations were observed for the chemical shift values of C-14, C-15, C-16, and C-20−C-25, altogether indicating that structural changes had occurred within the tryptophan ring system. All proton signals despite H-3 and H-18, which were absent in **2**, together with CH$_3$-16 seen as a singlet in **2**, could be assigned similarly in **1** and **2**.



Taken together, the above observations strongly indicated that **2** was formally derived from **1** by the formation of a bond between C-3 and C-17 and of a carbonyl group at C-18. This structural assignment was supported by HMBC results, including a correlation between the protons resonating at 1.19 ppm (H-16) and the quaternary carbon resonating at 54.7 ppm (C-17) (Table 1).

A NOESY experiment clarified the relative configuration around the spiro-center at C-17. Interaction between the protons resonating at 8.63 ppm (H-2) and 7.18 ppm (H-24) (Table 1) supported the depicted geometry of the tryptophan moiety. This geometry is identical to that reported in spiroquinazoline[7] (**5**). Takahashi et al. described a similar ring closure between C-3 and C-17 in fumiquinazoline C, via an ether linkage.[6]

Despite having very similar ring systems, the long-range proton−proton and proton−carbon couplings observed for **2** and **5** are different. For instance H-15

* To whom correspondence should be addressed. Tel.: +45 45 25 26 32. Fax: +45 45 88 49 22. E-mail: TOL@IBT.DTU.DK.
† Technical University of Denmark.
‡ Royal Danish School of Pharmacy.
§ University of Copenhagen.

S0163-3864(98)00056-1 CCC: $15.00
Published on Web 07/28/1998

Table 1. NMR Data for Alantrypinone (**2**) in CD_3CN-dmso-d_6 (10:1)

no.	^{13}C	^1H (mult, J = Hz)	HMBC	NOESY
1	169.5		14H, 15-CH_2, 16-CH_3	
2		8.63 br s		16H, 24H
3	61.9		2-NH, 16-CH_3,	
4	152.7		2-NH, 14H, 16-CH_3	
6	146.9		7H, 8H, 9H,10H	
7	127.2	7.68 ddd (7.1, 1.2, 0.5)	8H, 9H	8H
8	134.0	7.85 ddd (7.1, 7.1, 1.7)	9H, 10H	7H, 9H
9	126.5	7.58 ddd (7.1, 7.1, 1.2)	7H, 8H	8H, 10H
10	126.0	8.19 ddd (7.1, 1.7, 0.5)	8H, 9H	9H
11	120.2		7H, 9H	
12	158.4		7H, 9H, 10H, 14H	
14	51.8	5.58 ddd (3.6, 2.0, 2.0)	2-NH	15H
15	35.9	2.41 dd (14.4, 2.0)		14H, 24H
		2.53 dd (14.4, 3.6)		
16	12.8	1.19 s	2-NH	2-NH
17	54.7		14H, 15-CH_2, 16-CH_3, 19-NH, 24H	
18	176.6		15-CH_2, 19-NH	
19		9.84 s		21H
20	142.4		19-NH, 21H, 22H, 24H	
21	109.5	6.95 d (7.5)	22H, 23H	19H, 22H
22	128.8	7.31 td (7.5, 0.9)	24H	21H, 23H
23	122.0	7.11 td (7.5; 0.9)	21H	22H, 24H
24	123.6	7.18 dd (7.5, 0.9)	22H	2-NH, 15H, 23H
25	129.8		15-CH_2, 19-NH, 21H, 22H, 23H	

can be seen as a ddd in **2** due to coupling to both protons at C-15 together with a strong W coupling to NH-2, as seen in the H−H COSY spectrum, whereas H-15 is seen as a singlet in **5**. Likewise, more long-range couplings are observed within the two benzene ring systems of **2** than of **5**.

X-ray analysis was performed in order to establish the absolute configuration of **2** (Figure 1).[8] The assignment was based on anomalous scattering according to the procedure described by Flack.[9] The asymmetric unit consists of two crystallographically independent molecules of alantrypinone, two independent molecules of DMSO, and one molecule of CH_3CN. The two independent molecules of alantrypinone had nearly identical geometry and conformation, only the tryptophan ring system is oriented slightly differently. These two molecules are related to each other by a pseudotranslation axis along the *z*-axis ($z = 0.5$). One of the DMSO molecules is observed disordered, with sulfur observed in two different positions (occupation factors: 0.77, 0.23). Bond lengths and bond angles of alantrypinone are within the expected ranges.[10] In the crystal packing, hydrogen bonds are observed between alantrypinone and DMSO and between two independent molecules of alantrypinone (N−H···O).[11] Furthermore, attractive interactions are observed between solvent molecules. Residual densities close to the disordered DMSO molecules remain to be described.

The absolute configuration of alantrypinone is (3*R*) and (14*R*), suggesting that the peptide incorporates L-alanine and D-tryptophan, as do also fumiquinazoline F, fumiquinazoline C, and spiroquinazoline. We submit that modular peptide synthases[12,13] are responsible for the biogenesis of these compounds. The precursors are then L-alanine and L-tryptophan, but the configuration of tryptophan is reversed during an enzymatic reaction.

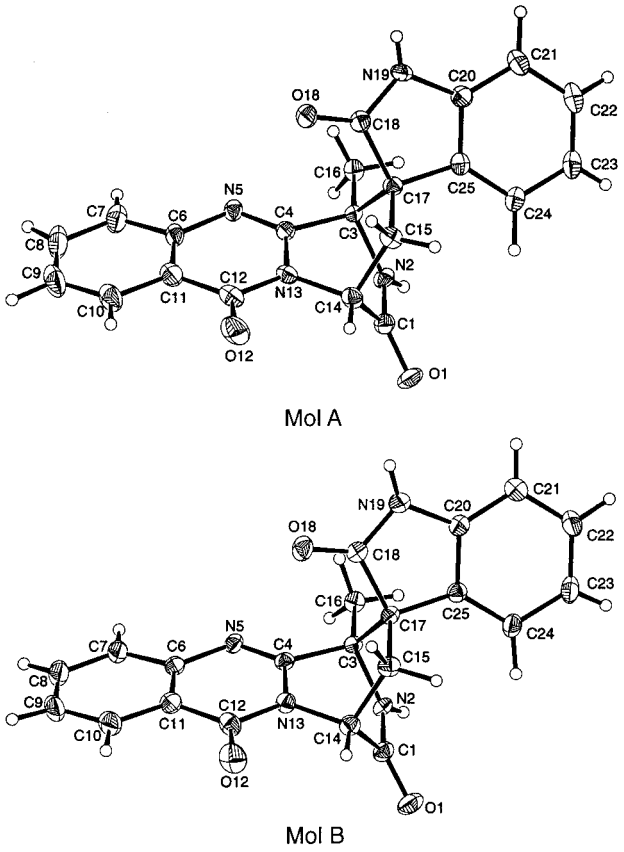


Figure 1. The molecular structure (ORTEPII[8]) of molecule A (upper) and molecule B (lower) of 2 show the absolute configuration. Atoms labeling scheme for nonhydrogen atoms is shown. Displacement ellipsoids of the nonhydrogen atoms are shown at the 50% probability level. Hydrogen atoms are represented by spheres of arbitrary size.

Experimental Section

General Experimental Procedures. NMR spectra were recorded in $CDCl_3$ (**1**) and in CD_3CN−DMSO-d_6 (10:1) (**2**) on a Varian 400 FT-NMR spectrometer at 400.0 and 100.6 MHz for ^1H and ^{13}C NMR spectra, respectively. 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.

Single-Crystal X-ray Crystallography of Compound Alantrypinone (2). X-ray diffraction data were collected on an Enraf−Nonius CAD-4 diffractometer with $\omega/2\theta$ scan mode, λ(Cu $K\alpha$) = 1.5418 Å. The crystal was cooled with an Enraf−Nonius low-temperature device. A suitable single crystal (0.40 × 0.12 × 0.10 mm) was obtained from a solution of the compound in CH_3CN−DMSO (10:1). This solvent mixture afforded crystals containing alantrypinone, DMSO, and CH_3CN [$C_{21}H_{16}N_4O_3$·$(CH_3)_2SO$·0.5 CH_3CN, M_w 471.03]; melting point 327 °C (morphological changes were observed during heating).

The crystal belongs to the trigonal system, space group $P3_2$ (no. 145) with $a = b = 17.242(3)$ Å, $c = 13.157(1)$ Å, $V = 3387.4(9)$ Å^3, Z = 6, $D_{calc} = 1.38$ g/cm^3 and μ(Cu $K\alpha$) = 1.619 mm^{-1}; $\theta_{max} = 74.8°$, $-21 \le h \le 21$, $-21 \le k \le 21$, $-16 \le l \le 16$. The 28 251 reflections collected were averaged according to the point group symmetry 3, resulting in 9298 unique reflections ($R_{int} = 0.0265$).

Data reduction was performed using DREADD.[14] Five standard reflections were measured every 10^4 s and indicated a systematic decay of 6.7% in the course of the data collection. Appropriate scaling was performed. Correction for absorption was performed ($t_{min} = 0.669$, $t_{max} = 0.876$).[15] The structure was solved by direct methods,[16] where all nonhydrogen atoms of alantrypinone were located. The nonhydrogen atoms of the solvent molecules, DMSO and CH_3CN, were found in subsequent difference electron density maps. One of the DMSO molecules was observed disordered with two different locations for the sulfur atom. Most of the hydrogen atoms were located in subsequent difference electron density maps. Refinement was performed by full-matrix least-squares procedure based on F^2 (SHELXL97).[17] Atomic coordinates and anisotropic displacement parameters were refined for nonhydrogen atoms. The positions of the hydrogen atoms of alantrypinone were refined with fixed isotropic displacement parameters. The hydrogen atoms of one of the DMSO molecules and the CH_3CN molecule were introduced in calculated positions and refined isotropically riding on their parent atom, using AFIX in SHELXL97. Final $R = 0.047$ and w$R(F^2) = 0.123$ for 8372 observed reflections ($I \ge 2\sigma(I)$) and 714 variables; $w = 1/[\sigma^2(F_o2) + (0.0908P)^2]$, $P = (F_o2 + 2F_c2)/3$; GooF = $S = 1.017$. The absolute configuration was established by refinement of the absolute structure parameter,[9] resulting in a Flack parameter $x = 0.00(2)$ for the (3*R*,14*R*)-enantiomer, shown in Figure 1. Atomic scattering factors were used as implemented in SHELXL97, taken from International Tables for Crystallography.[18] The following tables have been deposited: (a) fractional atomic coordinates and equivalent displacement parameters for nonhydrogen atoms; (b) fractional atomic coordinates for hydrogen atoms and isotropic displacement parameters; (c) anisotropic displacement parameters for nonhydrogen atoms; (d) bond lengths, bond angles, torsion angles, and close intermolecular interactions; and (e) observed and calculated structure factors.[11]

Fungal Material and Fermentation. The *P. thymicola* isolate (IBT 5891), originating from thyme, was obtained from the IBT Culture Collection at the Department of Biotechnology (IBT), Technical University of Denmark. The fungus was cultured for 14 days in 10 conical flasks (1 L) containing 200 mL of SYES liquid medium according to Svendsen and Frisvad,[19] without agar, however.

Extraction and Separation. The combined fungal mycelium was extracted twice with 300 mL of EtOAc and filtered through a Whatman 1PS phase separation filter before evaporation, to give approximately 500 mg of crude extract. This extract was subjected to vacuum−liquid chromatography[20] on silica (heptane, heptane−EtOAc, EtOAc−MeOH, MeOH as eluents) to give one fraction rich in alantrypinone and one rich in fumiquinazoline F. Each fraction was further purified on a Waters Prep Nova-Pak C-18 cartridge (25 × 100 mm, 6 μm, 60 Å) using 20 mL/min H_2O−CH_3CN (40:60) as mobile phase to give 18 mg of **1** and H_2O−CH_3CN (60:40) to give 22 mg of **2**.

Fumiquinazoline F (1): white powder; $[\alpha]^{22}_D$ −383° (*c* 2.50, $CHCl_3$); EIMS *m*/*z* 358 $[M]^{\cdot+}$ (47), 229 (8), 130 (100).

Alantrypinone (2): white amorphous solid; $[\alpha]^{22}_D$ +37° (*c* 2.08, EtOH); UV λ_{max} (EtOH) nm (log ϵ) 210 (3.78), 257 (3.15), 266 (3.17), 277 (3.08), 292 (2.73), 303 (2.77), 316 (2.62); CD (EtOH, *c* 0.024), $\Delta\epsilon$ (λ nm) 216 (+5.04), 229 (−1.69), 246 (+0.84), 274 (−0.75), 294 (+0.42), 326 (−0.14); EIMS *m*/*z* 372 $[M]^{\cdot+}$ (12), 227 (100), 199 (18), 145 (14), 117 (10); NMR, Table 1.

Acknowledgment. The assistance of Mr. Flemming Hansen in the X-ray intensity data collection is gratefully acknowledged. We are indepted to Dr. S. E. Harnung for determination of the CD data measured on a modified JASCO instrument financed by the Danish Natural Science Research Counsil, grant no.//-0373-1. The support of the Danish Dairy Foundation (Danish Dairy Board) and the Danish Research and Development Program for Food Technology is acknowledged.

References and Notes

(1) Larsen, T. O.; Frisvad, J. C.; Christophersen, C. *Biochem. Syst. Ecol.* **1998**, *26*, 463−465.
(2) Frisvad, J. C.; Filtenborg, O. *Mycologia* **1989**, *81*, 837−861.
(3) Svendsen, A.; Larsen, T. O.; Frisvad, J. C. Unpublished results.
(4) Penn, J.; Mantle, P. G.; Bilton, J. N.; Sheppard, R. N. *J. Chem. Soc., Perkin Trans.* 1 **1992**, 1495−1496.
(5) Yeulet, S.; Mantle, P. G. *J. Chem. Soc., Perkin Trans. 1* **1986**, 1891−1894.
(6) Takahashi, C.; Matsushita, T.; Doi, M.; Minoura, K.; Shingu, T.; Kumeda, Y.; Numata, A. *J. Chem. Soc., Perkin Trans. 1* **1995**, 2345−2353.
(7) Barrow, C. J.; Sun, H. H. *J. Nat. Prod.* **1994**, *57*, 471−476.
(8) Johnson, C. K. ORTEPII. Report ORNL-5138. Oak-Ridge National Laboratory, Tennessee, 1976.
(9) Flack, H. D. *Acta Crystallogr.* **1983**, *A39*, 876−881.
(10) International Tables for Crystallography. 1992, Vol C. Tab. 9.5.1.1.
(11) Atomic coordinates for this structure have been deposited with the CCDC and are available (free of charge) on request to the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK.
(12) Marahiel, M. A.; Stachelhaus, T.; Mootz, H. D. *Chem. Rev.* **1997**, *97*, 2651−2674.
(13) von Döhren, H.; Keller, U.; Vater, J.; Zocher, R. *Chem. Rev.* **1997**, *97*, 2675−2705.
(14) Blessing, R. H. *J. Appl. Crystallogr.* **1989**, *22*, 396−397.
(15) DeTitta, G. T. *J. Appl. Crystallogr.* **1985**, *18*, 75−79.

(16) Sheldrick, G. M. *Acta Crystallogr.* **1990**, *A46*, 467−473.
(17) Sheldrick, G. M. SHELXL97. Program for the refinement of crystal structures. University of Göttingen, Germany, 1997.
(18) International Tables for Crystallography; 1992, Vol C. Tab. 4.2.6.8. and 6.1.1.4.
(19) Svendsen, A.; Frisvad, J. *Mycol. Res.* **1994**, *98*, 1317−1328.
(20) Pelletier, S. W.; Chokshi, H. P.; Desai, H. K. *J. Nat. Prod.* **1986**, *49*, 892−900.

NP980056V