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Novofumigatonin, a New Orthoester Meroterpenoid from *Aspergillus novofumigatus*

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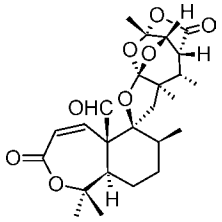
ABSTRACT



Novofumigatonin (1)

Novofumigatonin (1), a new metabolite, has been isolated from *Aspergillus novofumigatus.* The structure and relative stereochemistry were determined from HR ESI MS, one- and two-dimensional NMR, and single-crystal X-ray analysis. The absolute configuration was assigned using vibrational circular dichroism in combination with density functional calculations.

The pathogenic fungus *Aspergillus fumigatus* is known to produce a vast array of secondary metabolites. We have previously investigated a newly characterized species of this family named *A. novofumigatus*[1] and found a series of novel benzodiazepines, the *epi*-aszonalenins.[2] Further studies on this isolate have yielded an intriguing metabolite that was isolated and purified using UV-guided fractionation.

We here report the isolation and structure elucidation of the novel orthoester novofumigatonin (**1**), related to fumigatonin[3] from *A. fumigatus* (Figure 1). The isolate of *A. novofumigatus* (deposited in the IBT Culture Collection at BioCentrum-DTU, Technical University of Denmark as IBT 16806) was cultured on 200 YES agar Petri dishes at 25 °C for 14 days and extracted with ethyl acetate containing 1% HCO_2H.

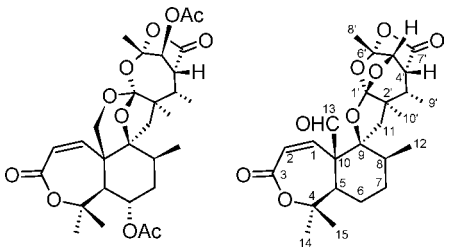


Figure 1. (Left) Structure of fumigatonin. (Right) Structure and numbering of novofumigatonin (**1**).

The agar plate extract (16.2 g) was chromatographed on flash reverse-phase columns (Phenomenex C-18, 50 μm) using a sharp, stepped gradient from water through to

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methanol. The fraction that eluted with 60% methanol (663 mg) was purified on a Waters column (300 mm × 19 mm, 15 μm, C-18), using 30 mL/min H_2O-CH_3CN (starting at 62:38, increasing to 24:76 over 60 min) as the mobile phase to yield **1** (120 mg). Novofumigatonin **1** was obtained as a white amorphous powder. The specific rotation was measured as $[\alpha]^{20}_D$ −148° (*c* 0.04, MeOH). The UV spectrum of **1** showed a maximum end absorption at 224 nm (log ϵ 5.03). The IR spectrum confirmed this observation by revealing three distinct absorption bands in the carbonyl region at 1796, 1782, and 1703 cm^{-1}. The ES positive ion mass spectrum of **1** showed strong $[M + H]^+$, $[M + CH_3CN + Na]^+$, and $[2M + Na]^+$ peaks at *m*/*z* 461, 525, and 943, respectively. High-resolution mass measurements on the $[M + H]^+$ ion gave *m*/*z* 461.2203, which, in combination with ^1H and ^{13}C NMR data, suggested a molecular formula $C_{25}H_{32}O_8$ (calcd 461.2175 for $C_{25}H_{33}O_8$).

A series of DQF-COSY, TOCSY, and gHSQC experiments established four spin-systems: **a**−**d** (Figure 2). The

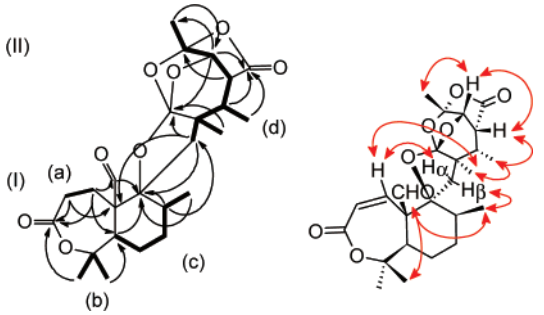

Figure 2. (Left) Important gHMBC correlations for fragments **I** (**a**, **b**, and **c**) and **II** (**d**) of novofumigatonin (**1**) in DMSO-d_6. The four spin-systems (**a**−**d**) are shown in bold. (Right) Important NOE correlations used to establish relative stereochemistry.

establishment of substructure **I**, comprised of spin-system **a**−**c**, was accomplished mainly from gHMBC correlations. Protons H1 and H2 both correlated to C3, as did methyl group H_3-14. The chemical shift values of C1, C2, and C4 clearly indicated that C3 is an ester carbonyl and that it was oriented with the oxygen attached to C4. H1 also showed a weak correlation to methine C5 as did H_3-14 and H_3-15. Finally, H2 correlated to the quaternary C10 and the aldehyde C13. H5 confirmed this with correlations to C4, C10, C13, and C15. H5 also correlated with the oxygen-substituted carbon C9, as did H13. H13 also correlated with C1 and C10. Spin-system **c** was elucidated as mentioned previously, and the gHMBC analysis confirmed this (see Table 1).

The rest of spin-system **c** was linked to the combined spin-systems **a** and **b** by the correlations from H8 and H_3-12 to C9 and H8 to C10, which confirmed the placement of the quaternary C9 and C10. A weak correlation was observed from H8 to the methylene C11 of spin-system **d**. Correlations were observed from H11 to C8, C9, and C10, which indicated placement of C11 as the linkage from fragment **I** to fragment **II**. The number of quarternary carbons in the area of this linkage, however, limited the number of gHMBC

Table 1. ^1H and ^{13}C NMR and HMBC Data for **1**[a]

no.	δ_C	δ_H (mult., *J*, Hz)	HMBC correlations
1	144.4	6.83, d, 13.1	2, 3, 5, 6, 9, 13
2	123.4	6.21, d, 13.1	1, 3, 10, 13
3	164.6	–	–
4	82.8	–	–
5	49.6	2.19, m	4, 6, 7, 9, 10, 13, 15
6α	18.7	2.14, qd, 13.2, 3.5	7, 10
6*β*		1.73, m	
7α	28.0	1.64, m	5, 6, 8, 9, 12
7*β*		1.86, m	
8	39.5	2.21, m	6, 7, 9, 10, 11, 12
9	90.0	–	–
10	62.2	–	–
11α	49.2	1.72, d, 13.8	8, 9, 10, 1′, 2′, 3′, 10′
11*β*		2.02, d, 13.8	
12	16.4	0.95, d, 7.2	7, 8, 9
13	201.4	10.26, s	1, 9, 10
14	30.2	1.35, s	3, 4, 5, 15
15	23.6	1.24, s	4, 5, 14
1′	128.1	–	–
2′	46.7	–	–
3′	38.0	2.04, m	11, 2′, 4′, 7′, 9′
4′	41.0	2.78, dd, 8.6, 6.2	2′, 3′, 5′, 6′, 7′
5′	77.6	5.01, d, 8.6	1′, 3′, 6′, 7′, 8′
6′	107.1	–	–
7′	175.1	–	–
8′	21.8	1.62, s	5′, 6′
9′	11.7	0.99, d, 7.1	2′, 3′, 4′
10′	18.5	0.75, s	11, 1′, 2′, 3′

[a] Data were recorded in DMSO-d_6 at 799.58 MHz for ^1H and 201.10 MHz for ^{13}C on a Varian Unity Inova spectrometer.

correlations. Spin-system **d** was elucidated with NMR, but the correct placement of the many oxygen bonds proved to be impossible to ascertain with NMR. Carbon C1′ was especially difficult to map.

A crystal was therefore grown in a crystallization tray from a saturated solution of 9:1 EtOH−H_2O. X-ray crystallographic analysis[4] revealed the remaining connectivities of **1** and also provided the relative stereogeometry as shown in Figure 3.

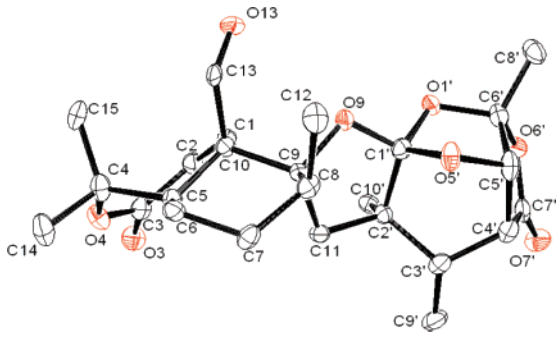

Figure 3. Three-dimensional structure of **1** acquired by X-ray crystallography with view point from C8 to C9. Carbon atoms in black and oxygens in red.

NOESY data for **1** (Figure 2) were consistent with the X-ray structure. The H5 proton correlated with both H_3-14

and H_3-15, but only H_3-15 correlated with H13. H_3-12 methyl group also correlated with H13. In combination with the X-ray structure, it was clear that C12, C13, and C15 were all positioned on the same side of the plane of the two rings in fragment **I**. H1 also correlated to one of the H11 protons (H11α) and H10′. H8 and H_3-12 correlated to the other (H11β), and a correlation between H_3-12 and H3′ was also observed. H_3-9′ and H_3-10′ correlated to each other, as did H3′ with H4′. Unfortunately, all attempts to cocrystallize with heavy atom compounds were unsuccessful; thus, a vibrational circular dichroism (VCD) study was undertaken to establish the absolute configuration of **1**. VCD has recently gained importance for the determination of absolute configuration of small organic molecules,[5] especially in those cases where X-ray crystallography is impossible (e.g., liquids[6]).

A VCD spectrum is obtained as the difference in absorbance when using left- and right circularly polarized light (in the infrared region). As a direct consequence hereof, enantiomers will have spectra which are mirror images of each other. For a typical small- to medium-sized molecule there will be numerous IR absorption modes (theoretically $3N - 6$, where N is the number of atoms), and the resulting VCD spectrum thus contains many absorptions which can be used to identify a particular compound. More importantly, however, the calculation of VCD spectra from first principles has matured to become a robust theoretical method which can be performed almost routinely, usually relying on density functional calculations (DFT) (B3LYP) and a relatively large basis set. This allows the determination of absolute configuration by simple comparison between experimental and theoretical spectra.

The computational investigation of **1** used the X-ray structure as the starting point, and after addition of hydrogens, the resulting structure was optimized using the OPLS-2005 force-field. This did not result in any significant changes of the structure (rms difference between the X-ray and the OPLS structure was 0.1758 Å, excluding hydrogens). Further structural optimization and calculation of optical properties (IR, VCD) was performed using density functional theory (B3LYP/6-31G**)[7,8] in Gaussian03.[9] Lorentzian lineshapes were assumed with a half-width of 4 cm^{-1} in the theoretical IR and VCD spectra, and a frequency scaling factor of 0.98 was used throughout. Due to the size and complexity of **1** a perfect peak-to-peak match between theoretical and experimental spectra cannot be expected.

Accordingly, we only attempted to compare the major features in IR. Given an adequate match, VCD could be used to assign the absolute configuration of **1**. The experimental measurements were carried out by Biotools Inc. The IR and VCD spectra were recorded at a resolution of 8 cm^{-1} over a period of 8 h, using a 100 μL solution of **1** (4.2 mg in $CDCl_3$).

Figure 4 shows the calculated and measured IR absorption spectra. The selected wavenumber range corresponds to

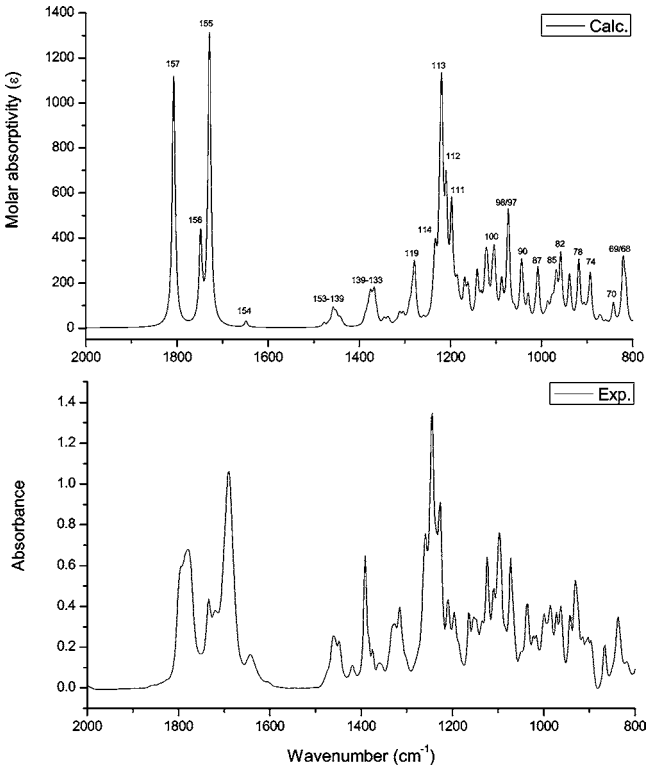

Figure 4. (Top) Calculated IR spectrum of novofumigatonin. (Bottom:) Experimental IR spectrum.

fundamentals 68−157 of the possible 189 for **1**. The carbonyl stretching region is significantly broader in the experimental spectrum, which may be related to coordinating solvent molecules. The inclusion of an implicit solvation model did not lead to improved results, and we deemed inclusion of explicit solvent molecules to be too computationally demanding. In the "fingerprint" region (1400−800 cm^{-1}) there are a multitude of vibrations associated with the skeleton of **1**.

The carbonyl stretching region did not produce useful VCD signals; thus, we have limited ourselves to the fingerprint region (1500−800 cm^{-1} see Figure 5). The two most prominent peaks in the calculated spectrum (Figure 5,

(4) CCDC 670389 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

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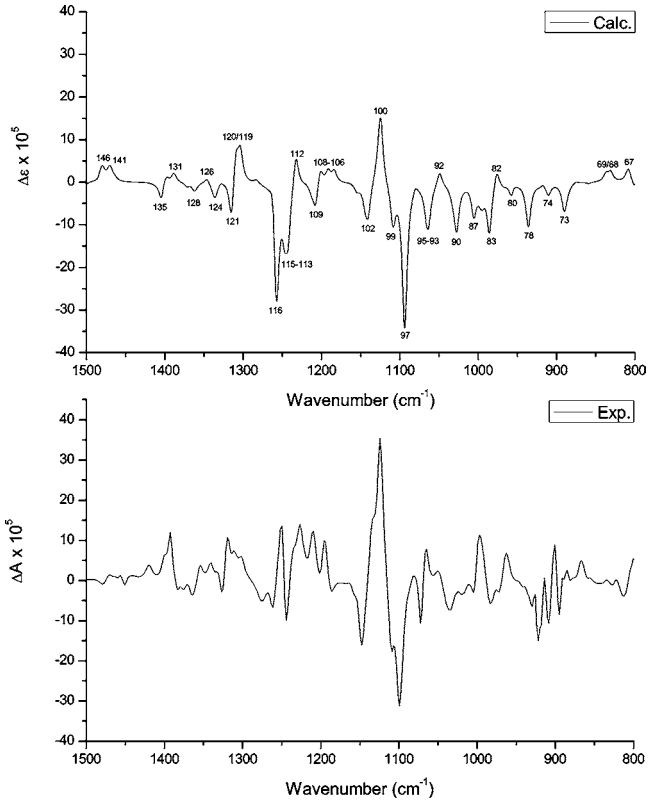

Figure 5. (Top) Calculated VCD spectrum of novofumigatonin. (Bottom) Experimental VCD spectrum.

top) are the positive peak resulting from fundamental no. 100 and the negative peak from fundamental no. 97.

These peaks can also be clearly identified in the experimental spectrum (Figure 5, bottom), and the good agreement allows assignment of the absolute configuration of **1** as shown in Figure 1. To assist further in the corroboration of this result we also note good agreement for peaks 102, 95−93, 92, and 90. The experimental spectrum is relatively poor in the region close to 900 cm^{-1}, which can be attributed to the strong absorption of the solvent ($CDCl_3$). Another discrepancy exists in the region close to 1250 cm^{-1}, where the calculation predicts large negative VCD absorptions (fundamentals 113−116), which cannot be found in the experimental spectrum.

A comparison of **1** to known compounds shows a high similarity to fumigatonin.[3] The major difference is the presence of an aldehyde substituent on C10 where fumigatonin has an oxygen-bound methine group and the absence of an acetate group at C6 on **1**. A closer comparison of spin-system **d** to the corresponding unit of fumigatonin also reveals that this part has the same relative stereochemistry, except for the acetate-bearing C5′ in fumigatonin. The major difference in the backbone between **1** and fumigatonin is in the linkage between fragments **I** and **II**.

The structure of fragment **I** also has some resemblance to the andilesins, fungal metabolites previously found in *Aspergillus variecolor*.[10,11] A closer comparison between fumigatonin and **1** reveals that all stereocenters have the same configuration, which strongly indicates a common biosynthetic route.

Simpson *et al.*[12] proposed that fumigatonin arises from a mixed polyketide-terpenoid pathway, similar to that of the andilesins and andibenins, but no work has been presented to date on this subject.

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Supporting Information Available: One- and two-dimensional NMR spectra of **1,** along with a full Gaussian03 reference (for ref 9), *XYZ* coordinates of the optimized structure, and enlarged IR and VCD spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

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