Available online at www.sciencedirect.com
SCIENCE DIRECT
Tetrahedron Letters
Tetrahedron Letters 47 (2006) 6099–6102

epi-Aszonalenins A, B, and C from *Aspergillus novofumigatus*

Christian Rank,[a,*] Richard Kerry Phipps,[a] Pernille Harris,[b] Jens Christian Frisvad,[a] Charlotte Held Gotfredsen[b] and Thomas Ostenfeld Larsen[a]

[a]*Center for Microbial Biotechnology, BioCentrum, Søltofts Plads, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark*
[b]*Department of Chemistry, Kemitorvet B207, Technical University of Denmark, DK-2800 Kgs. Lyngby, Denmark*

Received 5 May 2006; revised 5 June 2006; accepted 15 June 2006
Available online 7 July 2006

Abstract—Three new benzodiazepines have been isolated from an unusual chemotype of *Aspergillus novofumigatus*: *epi*-aszonalenins A, B, and C. The structures were elucidated by use of one- and two-dimensional NMR spectroscopic techniques and HR ESI MS. The relative configuration was established on the basis of a single crystal X-ray diffraction study of *epi*-aszonalenin A and the absolute configuration was determined by optical rotation comparison with the literature data. The absolute configurations of *epi*-aszonalenins B and C were determined by circular dichroism comparison to *epi*-aszonalenin A.

As part of our research on industrial or otherwise important human related filamentous fungi, we have been involved in the description of the new *Aspergillus* sp. named *Aspergillus novofumigatus*.[1] Despite being closely related to *A. fumigatus*, three of the major metabolites from *A. novofumigatus* turned out to be three new variants of the known fungal metabolite aszonalenin.[2–5] The metabolites were found by the combination of the knowledge of similar compounds and the intelligent X-hitting strategy based on UV-data developed in our group.[6,7] The analysis strongly indicated that the three new compounds had a benzodiazepine-like structural motif. This was intriguing as benzodiazepines are often found to have psychoactive properties and several members of this compound class are currently in use as psychoactive drugs.[8]

epi-Aszonalenins A (**1**), B (**2**), and C (**3**) (Fig. 1) were isolated from an organic extract of *A. novofumigatus* grown on yeast extract sucrose (YES) agar. The extract was fractionated using C-18 reverse phase (RP) vacuum liquid chromatography and the fractions containing target molecules were selected from analytical HPLC on the basis of UV spectra, and then fractionated by preparative RP-HPLC-DAD chromatography.

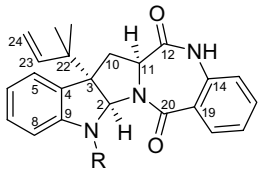

Figure 1. Structures of *epi*-aszonalenins A (**1**), B (**2**), and C (**3**) from *Aspergillus novofumigatus*.

A. novofumigatus (IBT16806) was cultured on 200 plates of YES agar at 25 °C for 14 days, and then extracted with ethyl acetate. The agar plate extract (16.2 g) was chromatographed on a Phenomenex C-18, 50 μm flash RP column using a sharp, stepped gradient from water to methanol in 10% steps. The fraction that eluted with 60% MeOH (0.66 g) was purified on a Waters HPLC column (300 × 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 a mixture of **1** and **2** (180 mg combined weight).

Compounds **1** and **2** were separated on a Phenomenex Luna C-18 column (250 × 10 mm, 5 μm, C-18) using 5 mL/min H_2O–CH_3CN (isocratic at 59:41 over 20 min) as the mobile phase to yield **1** (60 mg) and **2** (70 mg). Compound **3** was found in the fraction that

* Corresponding author. Tel.: +45 4525 2725; fax: +45 4588 4922; e-mail: cr@biocentrum.dtu.dk

0040-4039/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2006.06.086

eluted with 70% MeOH (1.38 g) and was further concentrated by fractionating through Sephadex LH-20 using MeOH as the eluent. A 50 mg portion of this extract was purified on a Phenomenex Luna C-18 column (250 × 10 mm, 5 μm, C-18) using 5 mL/min H_2O–CH_3CN (starting at 60:40, increasing to 45:55 over 15 min) as the mobile phase to yield **3** (9.5 mg).

epi-Aszonalenin A (**1**) was isolated as a white solid. The structure was elucidated by a combination of NMR spectroscopy and HR ESI mass spectrometry. The HR MS[9] of **1** was consistent with a molecular formula of $C_{25}H_{25}N_3O_3$.

The NMR data used for the structure elucidation of **1** were first acquired in DMSO-d_6 as the solvent. The ^{1}H NMR spectrum confirmed a benzodiazepine-like structure, as predicted by the X-hitting strategy. NMR-data of the compound in $CDCl_3$ were also obtained for *epi*-aszonalenin A to allow for comparison with the literature data.[3,4] The NMR-data obtained in $CDCl_3$ was less resolved than the NMR-data acquired in DMSO-d_6. The data for compounds **1** and **3** were almost the same as reported for acyl aszonalenin (acetyl substituent) and aszonalenin in 1982.[3] Furthermore gHMBC correlations confirmed connectivities in **1** as presented in Figure 2.

The proton shift values for H10a, H10b, and H11 in **1** however, were not the same as seen for acyl aszonalenin and more noteworthy, completely different $^3J_{HH}$ couplings were observed between the two H10's and H11 protons when compared to the literature data. Both the NMR-data from DMSO-d_6 and $CDCl_3$ supported these findings with almost identical values. Contrary to the two couplings of the known acyl aszonalenin, the H11 and one of the H10's of **1** show only one coupling: the doublet-H10 (designated H10a) had a coupling constant close to 13.3 Hz—a value typical of a geminal coupling. H10b is a doublet of doublets with a geminal coupling to H10a and a coupling to H11 of 9.5 Hz.

According to the Karplus equation,[10] the dihedral angle between H11 and H10a must approach ninety to minimize the *J* coupling constant, making the orbital overlap between C–H10a and C–H11 minimal. The H11 proton of aszonalenins has the opposite configuration at the bridgeheads C3 and C2 to the known aszonalenins.[2–5] Since the relative stereo geometry of the isoprene unit dictates that of C2, then H2 has to be on the same side of the two fused five-membered rings as the isoprene unit, the only variable was the configuration around C11. H11 had to point in the same direction as the isoprene unit. Figure 3 presents this difference in a Newman projection.

A series of NOE experiments were conducted in DMSO-d_6 to establish the stereo geometry of C11. Expected correlations between H11 and H2 were vaguely observed with mixing times above 400 ms, but this could originate from spin diffusion through H10b and the two methyl groups of the isoprene unit (C25 and C26).

X-ray crystallography supported the argument for **1** by providing a conformation for *epi*-aszonalenin A that was in agreement with the documented ^{1}H NMR coupling constants and hence different from acyl aszonalenin. The X-ray structure showed disorder around the C3–C22 bond, so that two different orientations of the isoprenoid moiety were present. Figure 4 presents one of these conformations.

epi-Aszonalenin B (**2**) was found to be a white solid and its HR MS[12] was consistent with a molecular formula of $C_{24}H_{23}N_3O_3$. NMR data revealed that the structure only varied at the substituent on N1. It contained a

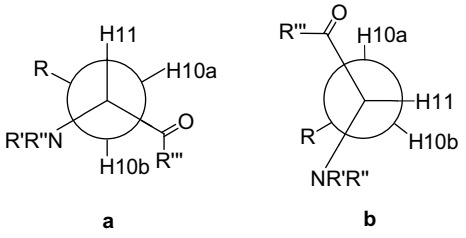

Figure 3. Newman projections of (a) C11–C10 of acyl aszonalenin and (b) C11–C10 of *epi*-aszonalenin A. (a) is a simplification of a MM2 model and (b) is based on the X-ray structure. In (a) the geometry of the protons on the two carbons will give rise to coupling between H11 and both of the H10's. The geometry presented in (b) will contrary to (a) give only one large coupling for H11–H10b (ideally), since in this configuration H10a is orthogonal to H11. The measured dihedral angles from X-ray analysis were: H11–H10a: 105.7° and H11–H10b: −9.9°.

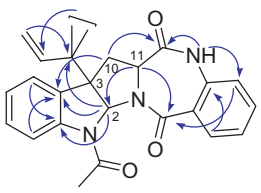

Figure 2. Important gHMBC correlations observed for compound **1**.

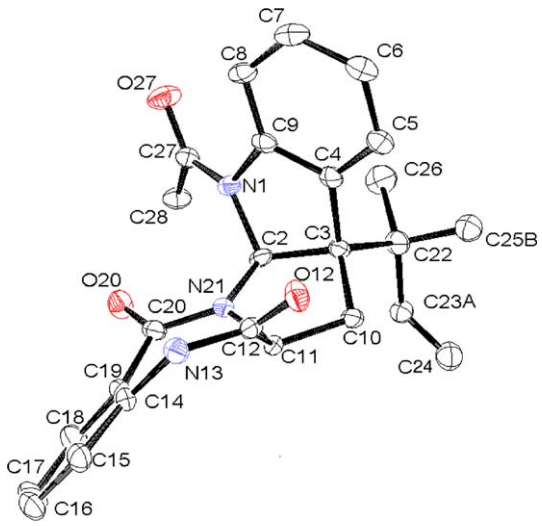

Figure 4. X-ray structure of *epi*-aszonalenin A.[11]

Table 1. NMR data of *epi*-aszonalenins A (**1**),[a] B (**2**),[b] and C (**3**)[b]

	epi-Aszonalenin A (**1**)		*epi*-Aszonalenin B (**2**)		*epi*-Aszonalenin C (**3**)	
	δ_H multiplicity (*J* in Hz)	δ_C	δ_H multiplicity (*J* in Hz)	δ_C	δ_H multiplicity (*J* in Hz)	δ_C
1	—	—	—	—	6.35 br s	—
2	6.05 s	80.9	6.27 s	78.5	5.55 s	78.9
3	—	60.6	—	60.3	—	60.4
4	—	132.9	—	133.2	—	129.4
5	7.45 br d (7.8)	127.3	7.47 br d (7.6)	126.5	7.12 d (7.6)	125.3
6	7.09 dt (7.8, 1.0)	123.3	7.13 dt (7.6, 1.0)	123.6	6.60 t (7.6)	116.6
7	7.19 dt (7.8, 1.0)	127.8	7.22 dt (7.6, 1.0)	127.9	6.93 t (7.6)	127.3
8	7.65 br d (7.8)	117.9	7.69 br d (7.6)	115.2	6.48 d (7.6)	108.2
9	—	141.5	—	139.3	—	149.2
10a	2.99 d (13.2)	28.9	2.96 d (13.3)	29.7	2.89 dd (13.5, 1.4)	31.1
10b	2.56 dd (13.2, 9.5)		2.58 dd (13.3, 9.4)		2.53 dd (13.5, 9.7)	
11	4.26 d (9.5)	56.7	4.36 d (9.4)	56.5	4.23 d (9.7, 1.4)	56.2
12	—	170.6	—	170.1	—	170.2
13	10.10 s	—	10.14 s	—	10.10 s	—
14	—	137.0	—	137.0	—	136.6
15	6.97 br d (7.9)	120.9	7.02 br d (7.9)	120.9	7.03 br d (7.8)	120.7
16	7.49 dt (7.9, 1.4)	132.7	7.51 dt (7.9, 1.4)	132.7	7.49 dt (7.8; 1.3)	132.1
17	7.20 br t (7.9)	123.6	7.23 dt (7.9, 1.0)	123.4	7.21 br t (7.8)	123.2
18	7.86 dd (7.9, 1.4)	130.8	7.85 dd (7.9, 1.4)	130.0	7.83 dd (7.8, 1.3)	130.0
19	—	124.6	—	124.6	—	125.4
20	—	166.3	—	166.1	—	165.1
22	—	40.2	—	40.9	—	41.1
23	5.91 dd (17.4, 10.7)	143.9	6.02 dd (17.3, 10.8)	143.3	6.08 dd (17.4, 10.5)	144.0
24	5.11 d (17.4)	113.9	5.09 dd (17.3, 0.8)	114.3	5.06 dd (17.4, 1.0)	113.3
	5.08 d (10.7)		5.11 dd (10.8, 0.8)		5.10 dd (10.5, 1.0)	
25	0.87 s	22.8	0.92 s	22.3	0.91 s	22.2
26	1.11 s	22.1	1.07 s	21.6	1.06 s	22.0
C=O	—	169.5	9.02 s	161.9		
CH_3	2.61 s	23.4				

[a] Acquired in DMSO-d_6 at 499.87 MHz (^{1}H) and 125.71 MHz (^{13}C), respectively.
[b] Acquired in DMSO-d_6 at 799.63 MHz (^{1}H) and 201.01 MHz (^{13}C), respectively. Both spectrometers were Varian Unity Inova. Spectra were referenced according to solvent resonances at $\delta_H = 2.50$ and $\delta_C = 39.43$ ppm, respectively. A line broadening of 0.3 Hz was applied to the ^{1}H NMR spectra.

formyl-substituent that was previously unknown for aszonalenins. Besides the change in N1-substituent, **2** had similar *J* couplings, and CD spectroscopic comparison with **1** showed almost identical data.

epi-Aszonalenin C (**3**) was also found to be a white solid and HR MS[13] indicated a molecular formula of $C_{23}H_{23}N_3O_2$, equivalent to the loss of CO compared to **2**. This was further supported by a simplification of the fine structure in the UV-spectrum for **3** when compared to the almost identical UV-spectra of **1** and **2**. NMR data again mostly varied around the N1-position and in this case there was no substituent. H11 of compound **3** showed an additional small coupling constant of 1.4 Hz, but this does not change the key point of the argument for the dihedral angles as shown in Figure 3.

It is plausible to expect that loss of the N1-substituent would give a larger change in the overall conformation, so the 90° dihedral angle between H11 and H10a was sufficiently obscured to give a small coupling. The changes in chemical shift values for both carbon and protons are also most noticeable in **3** compared to **1** and **2**. A comparison between the data of **3** to the data obtained by Bhat and Harrison in 1986[4] on (+)-dihydroaszonalenin (which is equal to **3** except for the saturation of the terpeniod double-bond) showed excellent coherence. The (+)-dihydroaszonalenin NMR data and optical rotation were almost identical to that of **3** and definitely different from (−)-dihydroaszonalenin and aszonalenin. CD spectroscopic comparison of **1**, **2**, and **3** confirmed that the overall stereochemistry was retained between the three.

Aszonalenins have themselves been shown to be substance P inhibitors for the human neurokinin-1 receptor[14] and one may expect a similar response for the *epi*-aszonalenin variants.

Apparently the aszonalenins are not produced by *A. fumigatus* or any of the other closely related species in section Fumigati, however, this is something that we will look further into, including the fully genome sequenced strain Af293.[15]

Acknowledgements

The spectra at 800 MHz were obtained on the Varian Unity Inova spectrometer of the Danish Instrument Center for NMR Spectroscopy of Biological Macromolecules. We acknowledge The Danish Research Councils for their support to our IVC *Center for Microbial Biotechnology* (2004–2008) (Grant No. 46-00-0005).

Supplementary data

1D ^{1}H NMR of **1**, **2**, and **3** has been supplied. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2006.06.086.

References and notes

1. Hong, S.-B.; Go, S.-J.; Shin, H.-D.; Frisvad, J. C.; Samson, R. A. *Mycologia* **2005**, *97*, 1316–1329.
2. Ellestad, G. A.; Mirando, P.; Kunstmann, M. P. *J. Org. Chem.* **1973**, *38*, 4204–4205.
3. Kimura, Y.; Hamasaki, T.; Nakajima, H. *Tetrahedron Lett.* **1982**, *23*, 225–228.
4. Bhat, B.; Harrison, D. M. *Tetrahedron Lett.* **1986**, *27*, 5873–5874.
5. Bhat, B.; Harrison, D. M. *Tetrahedron Lett.* **1993**, *49*, 10655–10662.
6. Hansen, M. E.; Smedsgaard, J.; Larsen, T. O. *Anal. Chem.* **2005**, *77*, 6805–6817.
7. Larsen, T. O.; Petersen, B. O.; Duus, J. Ø.; Sørensen, D.; Frisvad, J. C.; Hansen, M. E. *J. Nat. Prod.* **2005**, *68*, 871–874.
8. Medina, J. H.; Paladini, A. C.; Izquierdo, I. *Behav. Brain Res.* **1993**, *58*, 1–8.
9. *epi*-Aszonalenin A (**1**)—White powder; $[\alpha]_D^{20}$ +350 (*c* 0.8 mg/ml, $CHCl_3$) λ_{max} ($\log\varepsilon$) 220 (5.29) nm; HRESIMS: $m/z = 416.1988$ $[M+H]^+$, calcd for $[C_{25}H_{26}N_3O_3]^+$: 416.1974. Confirmed by adducts: $[M+Na]^+$ (438), $[M+MeCN+Na]^+$ (479), $[2M+Na]^+$ (853). NMR data are described in Table 1.
10. Haasnoot, C. A. G.; de Leeuw, A. M.; Allona, C. *Tetrahedron* **1980**, *36*, 2783–2792.
11. CCDC 609641 contains the Supplementary data for this letter. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
12. *epi*-Aszonalenin B (**2**)—White powder; $[\alpha]_D^{20}$ +386 (*c* 0.5 mg/ml, $CHCl_3$) λ_{max} ($\log\varepsilon$) 220 (5.47) nm; HRESIMS: $m/z = 402.1800$ $[M+H]^+$, calcd for $[C_{24}H_{24}N_3O_3]^+$: 402.1817. Confirmed by adducts: $[M+Na]^+$ (424), $[M+MeCN+H]^+$ (443), $[2M+Na]^+$ (825). NMR data are described in Table 1.
13. *epi*-Aszonalenin C (**3**)—White powder; $[\alpha]_D^{20}$ +670 (*c* 0.5 mg/ml, $CHCl_3$) λ_{max} ($\log\varepsilon$) 218 (5.46) nm; HRESIMS: $m/z = 374.1861$ $[M+H]^+$, calcd for $[C_{23}H_{24}N_3O_2]^+$: 374.1868. Confirmed by adducts: $[M+Na]^+$ (396), $[M+MeCN+H]^+$ (415), $[2M+Na]^+$ (769). NMR data are described in Table 1.
14. Barrow, C. J.; Sun, H. H. *J. Nat. Prod.* **1994**, *57*, 471–476.
15. Nierman, W. C.; Pain, A.; Anderson, M. J.; Wortman, J. R.; Kim, H. S.; Arroyo, J.; Berriman, M.; Abe, K.; Archer, D. B.; Bermejo, C.; Bennett, J.; Bowyer, P.; Chen, D.; Collins, M.; Coulsen, R.; Davies, R.; Dyer, P. S.; Farman, M.; Fedorova, N.; Fedorova, N.; Feldblyum, T. V.; Fischer, R.; Fosker, N.; Fraser, A.; García, J. L.; García, M. J.; Goble, A.; Goldman, G. H.; Gomi, K.; Griffith-Jones, S.; Gwilliam, R.; Haas, B.; Haas, H.; Harris, D.; Horiuchi, H.; Huang, J.; Humphray, S.; Jiménez, J.; Keller, N.; Khouri, H.; Kitamoto, K.; Kobayashi, T.; Konzack, S.; Kulkarni, R.; Kumagai, T.; Lafon, A.; Latgé, J.-P.; Li, W.; Lord, A.; Lu, C.; Majoros, W. H.; May, G. S.; Miller, B. L.; Mohamoud, Y.; Molina, M.; Monod, M.; Mouyna, I.; Mulligan, S.; Murphy, L.; O'Neil, S.; Paulsen, I.; Peñalva, M. A.; Pertea, M.; Price, C.; Pritchard, B. L.; Quail, M. A.; Rabbinowitsch, E.; Rawlins, N.; Rajandream, M.-A.; Reichard, U.; Renauld, H.; Robson, G. D.; Rodriguez de Córdoba, S.; Rodríguez-Peña, J. M.; Ronning, C. M.; Rutter, S.; Salzberg, S. L.; Sanchez, M.; Sánchez-Ferrero, J. C.; Saunders, D.; Seeger, K.; Squares, R.; Squares, S.; Takeuchi, M.; Tekaia, F.; Turner, G.; Vazquez de Aldana, C. R.; Weidman, J.; White, O.; Woodward, J.; Yu, J.-H.; Fraser, C.; Galagan, J. E.; Asai, K.; Machida, M.; Hall, N.; Barrell, B.; Denning, D. W. *Nature* **2005**, *438*, 1151–1156.