Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 941–943

TETRAHEDRON LETTERS

Absolute configuration of bioactive expansolides A and B from *Aspergillus fumigatus* Fresenius

Francisco A. Macías,[a,*] Rosa M. Varela,[a] Ana M. Simonet,[a] Horace G. Cutler,[b] Stephen J. Cutler[b] and Robert A. Hill[c]

[a]*Grupo de Alelopatía, Departamento de Química Orgánica, Universidad de Cádiz, Apdo. 40, Puerto Real (Cádiz), Spain*
[b]*Southern School of Pharmacy, Mercer University, 3001 Mercer University Drive, Atlanta, GA 30341-4155, USA*
[c]*HortResearch, Ruakura Research Centre, Ruakura, Hamilton, New Zealand*

Received 25 October 2002; revised 29 November 2002; accepted 4 December 2002

Abstract—In addition to antafumicins A and B, and cytochalasin, the bioactive compounds expansolides A and B have been isolated from *Aspergillus fumigatus* Fresenius. Their absolute configuration has been established by using the modified Mosher's method. The bioactivity of all isolated compounds has been evaluated.

Compounds from several different families have been isolated from the fungus *Aspergillus fumigatus*, mainly polyketides, but also sesquiterpenes, triterpenes, and steroids.[1] Diketopiperazines such as tryprostatins A and B, spirotryprostatins A, B, C and D and demethoxyfumitremorgin C and several known diketopiperazines such as fumitremorgin C have also been isolated from this fungus. All of these compounds are inhibitors of the mammalian cell cycle.[2]

During the course of examining fungi for biologically active natural products, we accessed a strain of *A. fumigatus* Fresenius from leaf litter collected in the Waipoua Forest, New Zealand. Five compounds were isolated from fermented *A. fumigatus* extract, grown in semisolid, still and shake fermentation,[3] using an etiolated wheat coleoptile bioassay to detect activity in the extracts, and to direct fractionation. These were the expansolides A (**1**) and B (**2**), previously isolated from *Penicillium expansum*;[4] antafumicins A (**3**) and B (**4**), previously isolated from *Aspergillus niger* NH-401;[5a,b] and cytochalasin E (**5**), previously isolated from various *Aspergillus* species[5c] (Fig. 1).

Compounds **1** and **2** were isolated as a mixture. Each of them, obtained as a pure compound by column chromatography, spontaneously gave rise to a mixture, in

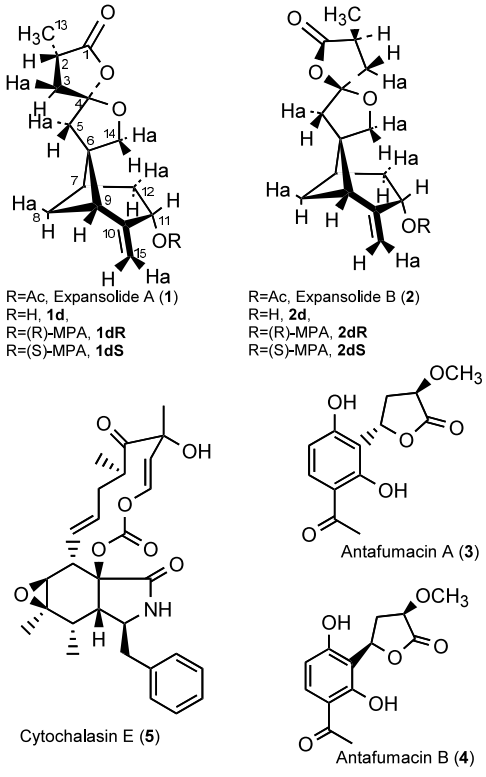


Figure 1. Structures of compounds **1**–**5** isolated from *A. fumigatus* Fresenius.

* Corresponding author. Tel.: +34-956-01-6370; fax: +34-956-01-6193; e-mail: famacias@uca.es

0040-4039/03/$ - see front matter
PII: S0040-4039(02)02778-8

various proportions, of both compounds when maintained in solution. Although these compounds have been previously described, with their relative configuration, we have now determined their absolute configuration, using the modified Mosher's methods. This method requires a secondary alcohol in the molecule, so we first saponified the mixture of **1**,**2** to obtain the 11-deacetyl derivatives **1d**,**2d**.[6] Then, the mixture was esterified with the enantiomers of the chiral reagent, methoxyphenylacetic acid (MPA),[7] which were subjected to HPLC separation, affording the compounds **1d***R*, **1d***S*, **2d***R* and **2d***S*.

The two conformations shown in Fig. 2 depict the preferred conformations of the esters **1d***R*, **2d***R*, **1d***S* and **2d***S*, if it is assumed that the C–O bond of the methoxy group is aligned with the C=O double bond of the carbonyl group.[8] Fig. 2 reveals that the positions β′, γ′ and δ′ will be juxtaposed with the phenyl group in the *R* esters, consequently the signals of the protons in these positions would be found upfield in the spectrum of the esters (*R*)-MPA relative to the signals for the (*S*)-MPA. For these protons the $\Delta\delta$ (*R*,*S*) will be negative. The reverse would be the case for the positions β, γ and δ.

The method requires the assignments of as many proton signals as possible of (*R*)- and (*S*)-MPA esters, to obtain the $\Delta\delta$ (*R*,*S*) values. Afterwards, protons with positive $\Delta\delta$ should be placed on the right-hand side, and those with negative $\Delta\delta$ values on the left-hand side of model A[9] (Fig. 2). The application of the model shows that the absolute configuration of C-11 of **1** and **2** is *S*, so the correct absolute configuration for expansolides A and B is (2*S*,4*S*,6*S*,7*R*,9*R*,11*S*) and (2*S*,4*R*,6*S*,7*R*,9*R*,11*S*), respectively.

The etiolated wheat coleoptile bioassay is rapid (<24 h) and is sensitive to a wide range of bioactive substances including plant growth regulators, herbicides,[10] antimicrobials, mycotoxins and assorted pharmaceuticals.[11] Crude extracts from an *Aspergillus* sp. showed high degrees of bioactivity in the etiolated wheat bioassay. Extracts from semisolid fermentation produced 100% inhibition in the bioassay, while shake and liquid fermentation extracts produced 80% inhibition.

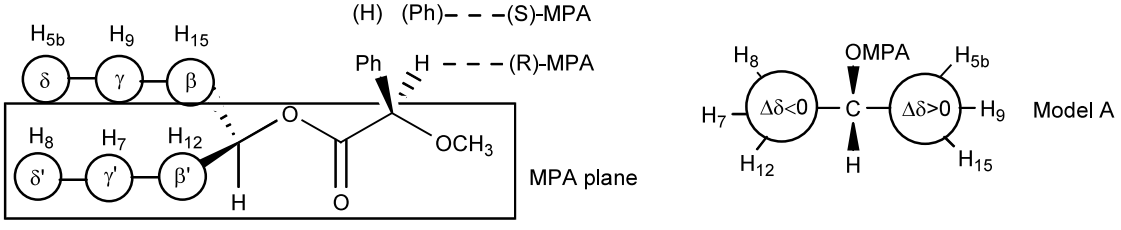
Etiolated wheat coleoptiles growth was significantly (p <0.01) inhibited by 100% with 10^{-3} M and 59% with 10^{-4} M solution of **1** and **2**. Compounds **3** and **4** showed 96% inhibition at 10^{-3} M, and the methylated derivatives of **3** and **4** provoked 80% inhibition at 10^{-3} M and 59% at 10^{-4} M. These values would be correlated with the obtained results[12] for the commercial herbicide LOGRAN,® generally used as internal standard, which showed 80% of inhibition at 10^{-3} M, and 42% of inhibition at 10^{-4} M, with no significative values with low concentration.

The inhibition by compound **5** was 57% at 10^{-3} M, all relative to controls. However, with this last product the sections were curved and resembled the half section of a coiled spring. This is an unusual response, but has been noted with indole-3-acetic acid (and other indolic compounds), and with some cytochalasins.[13]

Generally, with the cytochalasins,[13b] the sections showed banana shaped, with truncated tapering ends and fatter mid sections. There was also some slight curving of sections at 10^{-4} M, but there was no inhibition relative to the control. This response, without inhibition, is interesting and has been noted with the cytochalasins, but not with indole-3-acetic acid. This fact can be related with a different mode of action.

Acknowledgements

This research was supported by the Ministerio de Ciencia y Tecnología, Spain (MCYT; Project No. AGL-2001-3556). R.M.V. acknowledges a post-doctoral fellowship from Junta de Andalucía, Spain.



Δδ (R-S)	8a	8b	7	12a	12b	15a	15b	9	5b
1d	--	--	-0.06	-0.1	-0.12	+0.11	+0.09	+0.07	+0.12
2d	-0.01	-0.05	-0.07	-0.09	-0.15	+0.1	+0.07	+0.07	--

Figure 2. Configurational correlation model for the (*R*)- and (*S*)-MPA derivatives. The MPA plane is shown, H-15,9,5b and H-12,7,8 are on the right and left sides of the plane, respectively. Model A, to determine the absolute configuration at C-11, is illustrated.

References

1. Turner, W. B.; Aldridge, D. C. *Fungal Metabolites II*; Academic Press: London, 1983.
2. Cui, C.; Kakeya, H.; Okada, G.; Onose, R.; Osada, H. *J. Antibiot*. **1996**, *49*, 527–533.
3. (a) Cutler, H. G. In *Advances in Allelopathy*; Putman, A. C.; Tang, C. S., Eds.; John Wiley & Sons: New York, 1986; pp. 147–170; (b) *Fungal material*: The fungus was cultured on potato dextrose agar slants at 26°C for 10 days and was then maintained at 5°C until transfer to bulk fermentations.
   Semisolid fermentation: The fungus was transferred to Fernbach flasks (2.8 L), each containing 100 g of shredded wheat, 200 ml of Difco mycological broth (pH 4.8), 2% yeast extract, and 20% sucrose for metabolite production. Inoculated flasks were incubated in the laboratory at 24°C for 19 days.
   Still fermentation: The fungus was transferred to Fernbach flasks (2.8 L), each containing 200 ml of potato dextrose broth. Inoculated flasks were incubated in the laboratory at 24°C for 19 days.
   Shake fermentation: Identical preparation as in still fermentation, but subject to rotary shake at 80 rpm.
4. Massias, M.; Rebuffat, S.; Molho, L.; Chiaroni, A.; Riche, C.; Bodo, B. *J. Am. Chem. Soc*. **1990**, *112*, 8112–8115.
5. (a) Fujimoto, Y.; Miyagawa, H.; Tsurushima, T.; Irie, H.; Okamura, K.; Ueno, T. *Biosci. Biotechnol. Biochem*. **1993**, *57*, 1222–1224; (b) Fujimoto, Y.; Ukita, T.; Miyagawa, H.; Tsurushima, T.; Irie, H.; Nishimura, K.; Ueno, T. *Biosci. Biotechnol. Biochem*. **1994**, *58*, 1627–1631; (c) Büchi, G.; Kitaura, Y.; Yuan, S. S.; Wright, H. E.; Clardy, J.; Demain, A. L.; Glinsukon, T.; Hunt, N.; Wogan, G. N. *J. Am. Chem. Soc*. **1973**, *95*, 5423–5425.
6. *Deacetylated expansolides A (**1**) and B (**2**)*: The mixture **1**–**2** (10 mg) was dissolved in 1 mL of methanol and 2 mL of 0.05 M sulfuric acid and stirred continuously at 80°C for 1 h. Following the usual work-up, the product was purified using HPLC with an Si 60 5 μm column and eluted with *n*-hexane:EtOAc 3:2, using 1 mL min^{-1} flow rate and an RI detector yielding 7 mg of hydrolyzed product.
   Compound **1d**: δ_H (400 MHz, C_6D_6): 0.97 (3H, d, $J=7.2$ Hz, H-13), 1.36 (1H, dd, $J=12.8$, 11.4 Hz, H-3b), 1.63 (1H, d, $J=13.4$ Hz H-5b), 1.67 (1H, ddd, $J=15.4$, 3.2, 3.1 Hz, H-12b), 1.72 (1H, d, $J=9.7$ Hz, H-8b), 1.93 (2H, m, H-8a and H-12a), 1.98 (1H, dd, $J=12.8$, 8.3 Hz, H-3a), 2.07 (1H, d, $J=13.4$ Hz, H-5a), 2.30 (1H, m, H-7), 2.39 (1H, dd, $J=5.6$, 5.6 Hz, H-9), 2.61 (1H, ddq, $J=11.4$, 8.3, 7.2 Hz, H-2), 3.41 (1H, d, $J=9.7$ Hz, H-14b), 3.62 (1H, d, $J=9.7$ Hz, H-14a), 3.84 (1H, brd, $J=6.8$ Hz, H-11), 4.64 (1H, dd, $J=1.3$, 1.3 Hz, H-15b), 4.77 (1H, dd, $J=1.3$, 1.3 Hz, H-15a).
   Compound **2d**: δ_H (400 MHz, C_6D_6): 1.14 (3H, d, $J=7.4$ Hz, H-13), 1.49 (1H, d, $J=13.3$ Hz, H-5a), 1.63 (1H, dd, $J=13.3$, 5.8 Hz, H-3b), 1.75 (3H, m, H-3a, H-7 and H-12b), 1.80 (1H, d, $J=9.9$ Hz, H-8b), 1.94 (H-2, m, H-8a and H-12a), 2.07 (1H, d, $J=13.3$ Hz, H-5b), 2.19 (1H, ddq, $J=9.3$, 5.8, 7.4 Hz, H-2), 2.94 (1H, dd, $J=5.6$, 5.4 Hz, H-9), 3.36 (1H, d, $J=9.6$ Hz, H-14b), 3.64 (1H, d, $J=9.6$ Hz, H-14a), 3.86 (1H, brd, H-11), 4.75 (1H, brs, H-15b), 4.78 (1H, brs, H-15a).
7. Laypov, S. K.; Seco, J. M.; Quiñoá, E.; Riguera, R. *J. Org. Chem*. **1996**, *61*, 8569–8577.
8. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc*. **1973**, *95*, 512–519.
9. Ohtani, I.; Takenori, K.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc*. **1991**, *113*, 4092–4096.
10. Cutler, H. G. In *The Proceedings of the 11th Annual Meeting of the Plant Growth Regulator Society of America*; 1984, pp. 1–9.
11. Jacyno, J. M.; Cutler, H. G. *PGRSA Q*. **1993**, *21*, 15–24.
12. Castellano, D. Ph.D. Dissertation, Universidad de Cádiz, 2002.
13. (a) Wells, J. M.; Cutler, H. G.; Cole, R. J. *Can. J. Microbiol*. **1976**, *22*, 1137–1143; (b) Cutler, H. G.; Grumley, F. G.; Cox, R. H.; Cole, R. J.; Dorner, J. W.; Springer, J. P.; Latterell, F. M.; Thean, J. E.; Rossi, A. E. *J. Agric. Food Chem*. **1980**, *28*, 139–142.