

Notes

FUMIFUNGIN, A NEW ANTIFUNGAL
ANTIBIOTIC FROM *ASPERGILLUS*
FUMIGATUS FRESENIUS 1863

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From the metabolites present in the culture filtrate during the fermentation of a fungal culture, number Y-83,0405 and identified as *Aspergillus fumigatus* Fresenius 1863, a novel antifungal antibiotic, designated as fumifungin (**1**) was isolated. In this paper we report the isolation and structure elucidation of fumifungin.

The organism was isolated from a Himalayan soil sample. A few loopfuls of the well sporulated culture was used to inoculate 100 ml of the seed culture medium (soluble starch 1.5%, soybean meal 1.5%, yeast extract 0.2%, corn-steep liquor 0.1%, glucose 0.5%, CaCO₃ 0.2%, NaCl 0.5% in demineralized water at pH 6.5) distributed in 500-ml Erlenmeyer flasks. The seed culture was grown on a rotary shaker (240 rpm) at 26°C (±1°C) for 60 hours.

After a good growth was obtained it was used to inoculate (1%) 200 ml of the production medium (glucose 1%, malt extract 2%, peptone 1%, Na₂HPO₄ 0.1%, ZnSO₄·7H₂O 0.000022%, CaCl₂ 0.000055%, MnCl₂·4H₂O 0.00005%, FeSO₄·5H₂O 0.000016%, CoCl₂·6H₂O 0.000016% in demineralized water at pH 6.5) in 1-liter Erlenmeyer flasks. The fermentation was carried out on a rotary shaker (220 rpm) at a temperature of 26°C (±1°C) for 90 hours.

The culture filtrate (55 liters) at pH 5.9 was percolated through a Diaion HP-20 (1.8 liters) column. The resin bed was washed with de-

mineralized water (7 liters) followed by 50% aq MeOH (20 liters) and then eluted with MeOH-H₂O (4:1) until no more activity could be detected in the eluates (10 liters). Concentrated active eluates (7 liters) were diluted with water (15 liters) and recharged on Diaion HP-20 (1 liter). The resin bed was washed with 60% MeOH in water (10 liters) and then eluted with MeOH (4 liters). Removal of solvent gave crude fumifungin (1.5 g).

This crude material (0.6 g) was subjected to chromatography on reverse phase (octadecylsilyl) material (50 μ, 3.7×40 cm) at a flow rate of 11 ml/minute. The column was eluted with 60% MeOH in water (1 liter) and then with 70% MeOH in water when fumifungin eluted out. The active fractions were concentrated under reduced pressure and then lyophilized to obtain pure fumifungin (0.1 g).

Fumifungin (**1**) is a colorless solid, mp 108°C soluble in DMSO, MeOH and aq NaHCO₃. It decolorizes dilute KMnO₄ solution and gives violet coloration with ninhydrin. Fumifungin has a molecular weight of 431 (fast atom bombardment mass spectrum (FAB-MS) *m/z* 432 (M+H⁺)) and molecular formula C₂₂H₄₁NO₇ (high resolution electron impact mass spectra (HREI-MS) of the pertrimethylsilylated fumifungin: M' = M + 5TMS, *m/z* 791.4860, the fragmentation pattern is shown in Scheme 1). Elemental analysis obtained C 58.90, H 9.54, N

Scheme 1. Mass spectral fragmentation (HREI-MS) of pertrimethylsilylated fumifungin (**1**) showing structurally significant fragment ions.

a = *m/z* 706.3879, b = *m/z* 399.3119, c = *m/z* 392.1751, d = *m/z* 674.4490, e = *m/z* 187.1520, f = *m/z* 573.3821, g = *m/z* 218.1035, R = Si(CH₃)₃.

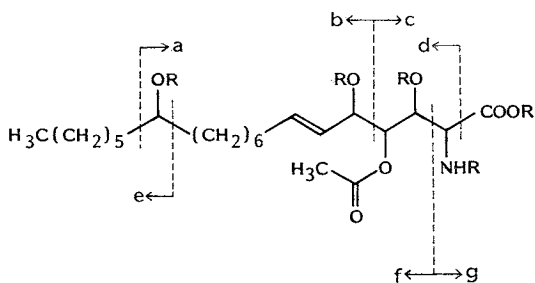
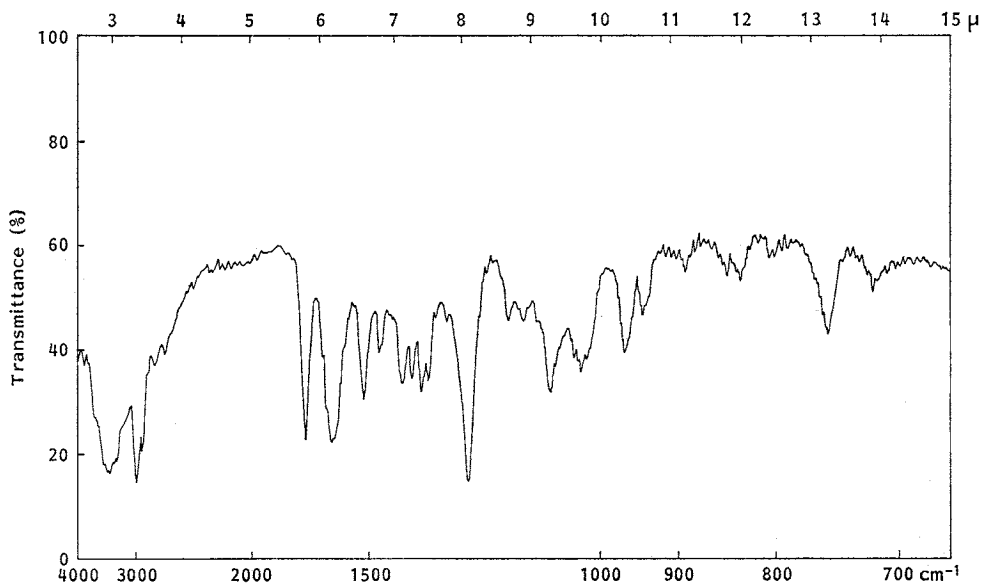
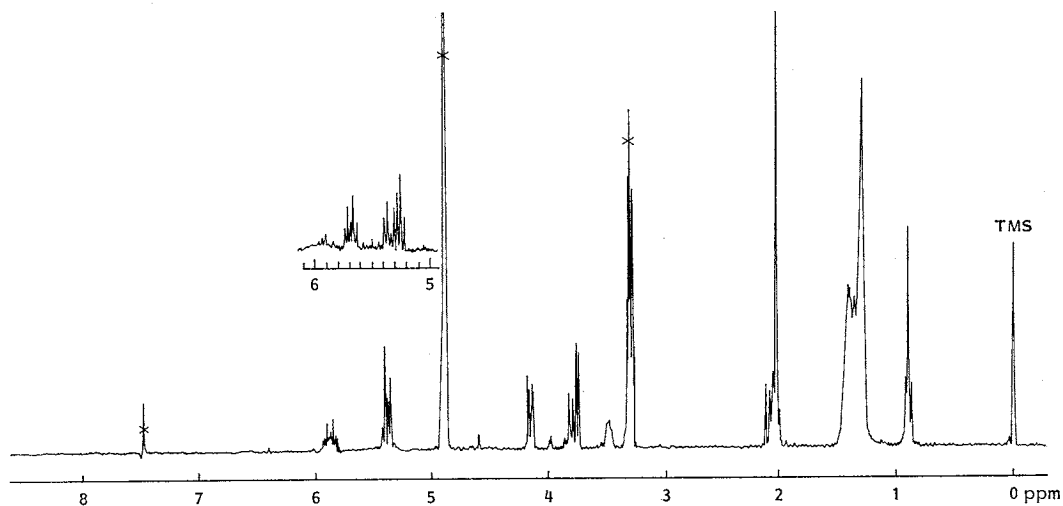


Fig. 1. IR spectrum (KBr, Perkin-Elmer 157) of fumifungin (1).

Fig. 2. 270 MHz ^1H NMR spectrum (CD_3OD , Bruker AM-270) of fumifungin (1); inset shows the 270 MHz ^1H NMR spectrum ($\text{DMSO}-d_6$) of the olefinic protons.

5.80~5.95 (1H, m), 5.32~5.45 (2H, m), 4.16 (1H, dd, $J=2$ and 4.5 Hz), 3.82 (1H, d, $J=6$ Hz), 3.76 (1H, d, $J=4.5$ Hz), 3.50 (1H, br s), 1.98~2.13 (5H, s and m overlapping), 1.20~1.50 (20H, m), 0.88 (3H, t, $J=6$ Hz).

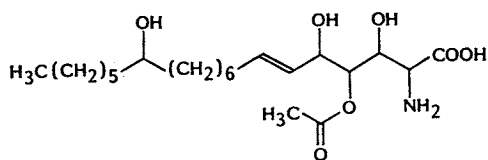


3.21; calcd for $\text{C}_{22}\text{H}_{41}\text{NO}_7$: C 61.25, H 9.51, N 3.20.

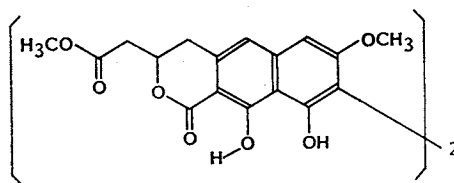
Fumifungin does not have any characteristic UV absorption in MeOH. Its IR and ^1H NMR spectra are shown in Figs. 1 and 2, respectively. The ^{13}C NMR spectrum (22.5 MHz, $\text{DMSO}-d_6$) showed the following signals: δ 169.37 (s), 169.05 (s), 134.80 (d), 125.38 (d), 75.67 (d), 71.95 (d),

69.46 (d), 68.27 (d), 56.24 (d), 37.17, 31.64, 31.32, 28.82, 28.50, 28.28, 25.14, 22.00, 21.02 and 13.76 (q).

An analysis of all the data indicate that there are (a) two carbonyl groups — one carboxylic acid and an acetate, (b) a $-\text{CH}_2\text{CH}^E\text{CHCH}-$ group, (c) a $-\text{CHCH}(\text{O}-)\text{CH}-$ group, (d) a total of four CH-O groups and one CH-N group, (e)



1



2

Table 1. Antifungal activity of fumifungin (1).

Microorganism	MIC values ($\mu\text{g/ml}$)
<i>Candida albicans</i>	62.5
<i>Saccharomyces cerevisiae</i>	62.5
Wild yeast	62.5
<i>Aspergillus niger</i>	7.8
<i>Penicillium digitatum</i>	7.8
<i>Trichophyton mentagrophytes</i>	15.6
<i>Botrytis cinerea</i>	150
<i>Fusarium culmorum</i>	250
<i>Alternaria solani</i>	31.2
<i>Cercospora beticola</i>	0.9
<i>Cladosporium resinae</i>	0.9
<i>Piricularia oryzae</i>	125

a CH_2CH_3 group and (f) about ten CH_2 groups present in fumifungin. Interpretation of the high resolution mass spectral fragmentation as shown in Scheme 1 established the structure of fumifungin as 2-amino-4-acetoxy-3,5,14-trihydroxy- Δ^6 -eicosenoic acid. As can be seen from the structure 1, fumifungin is a long chain amino acid and is similar to thermozyiocidin¹⁾/myriocin²⁻⁴⁾ in its chemical structure. Stereochemistry of the chiral centers of fumifungin have not been determined.

From the culture filtrate a known antibacterial antibiotic, SC-28762/viriditoxin (2)⁵⁾, has also been isolated.

MIC values of fumifungin (1) against some selected fungi are shown in Table 1.

Acknowledgments

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