Note

Structures of Antafumicins A and B, Novel Antifungal Substances Produced by the Fungus Aspergillus niger NH-401

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Novel antifungal substances, antafumicins A and B, were isolated from a culture of Aspergillus niger NH-401 and determined to be trans- and cis-4-(3-acyl-2,6-dihydroxyphenyl)-2-methoxy-4-butanolide, respectively, by spectroscopic and single crystal X-ray analyses.

Aspergillus fungi are well known for producing various kinds of biologically active compounds, including antifungal and antibacterial agents.1 In our series of studies to search for antifungal substances of fungal origin, we found a pair of novel compounds in the culture medium of an isolate of Aspergillus niger (NH-401), which showed potent inhibitory activity against the spore germination of several phytopathogenic fungi, as well as against the growth of some bacteria. The compounds are considered to be stereoisomers with the same molecular formula based on structural studies, and have thus been designated as antafumicins A (1) and B (2), respectively.

The fungus was cultured on potato-sucrose-agar plates (90 mm dia., 500 plates) at 27°C in the dark for 10 days, and the culture plates were then extracted with acetone. Compounds 1 and 2 were detected in the extract by silica gel TLC-autobiography, using Colletorichum lagenarium as the testing subject. The extract was then concentrated in vacuo to an aqueous solution, which was successively extracted with ethyl acetate. The ethyl acetate extract, after being concentrated, was chromatographed in a silica gel column (silica gel 60, Merck), eluting with a solvent system of n-hexane-ethyl acetate. The eluate with the solvent ratio of 1:1, showing the antifungal activity, was concentrated, and the residue was purified by HPLC (Nucleosil 50-5, 7.5 dia. x 500 mm), eluting with a solvent system of n-hexane-ethyl acetate-methanol (550:450:1) at a flow rate of 2 ml/min. Almost equal amounts (ca. 10 mg) of 1 (tR 24 min) and 2 (tR 25 min) were respectively obtained as yellowish amorphous material.

Purified 1 and 2 were subjected to conventional spectral analyses. 1: [α]D = +45° (c = 0.004 in n-hexane-ethyl acetate-methanol (550:450:1)); HR-MS m/z: 266.0796 (M+; calcd. for C13H14O6, 266.0786); EI-MS m/z: 266 (M+), 234, 222, 189, 175, 152; IR (KBr) cm⁻¹: 3440, 3100, 1769, 1618; UV λmax(MeOH) nm (ε): 217 (21000), 279 (15500), 317 (8720). 2: [α]D = 0° (c = 0.004 in n-hexane-ethyl acetate-methanol (550:450:1)); HR-MS, EI-MS, IR, and UV were practically identical with those of 1. 1H- and 13C-NMR signals observed for 1 and 2 are listed in Table I, the correlations between the 1H- and 13C-NMR signal being observed by C-H COSY experiments. The molecular formula of both 1 and 2 was established as C13H14O6 based on HR-El-MS data (M+; m/z 266.0796) and by taking account of their 13C- and 1H-NMR spectra. Because the EI-MS data for 1 and 2 were essentially identical, and the NMR spectra were similar to each other, 1 and 2 are considered to be a pair of stereoisomers.

The presence of a γ-lactone moiety was suggested by the IR (νmax 1769 cm⁻¹) and the 1H-NMR (C-1) data, which is consistent with the >C(2)H-C(3)H-C(4)H γ-moiety deduced from the H-C and H COSY experiments. In addition to the lactone ring protons, the 1H-NMR spectrum indicated the presence of acetyl methyl protons (H-12), methoxy methyl protons (H-13) and two ortho-coupled aromatic protons (H-7 and H-8), these observations being compatible with the signals in the 13C-NMR spectrum. The acetyl group appeared to be connected to the benzene ring, because of its rather low νC=O absorption wave number in the IR spectrum at 1618 cm⁻¹. In the 1H-NMR spectrum, one hydrogen-bonded hydroxyl proton (10-0H) was also observed in both 1 and 2.

The gradual interconversion and/or decomposition of 1 and 2

![Image 1](image1.png)

Fig. 1. Proposed Structures of Antafumicins A (1), B (2), and Their Methylation Products 1-Me and 2-Me.

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in a solution hindered further structural studies by spectral analyses. The stability of the compounds, however, was found to be much improved by converting with diazomethane. The treatment with diazomethane gave monomethyl derivatives of 1 and 2 (1-Me and 2-Me), which was confirmed by EI-MS (M+; m/z 280) and 1H-NMR (new singlet signals observed at δ 3.89 in 1-Me and at 3.90 in 2-Me). Both hydrogen-bonded hydroxyl groups in 1 and 2 were found to remain unreacted (δOH 13.1) during this treatment, which clarifies the presence of an additional acidic (phenolic) hydroxyl group in 1 and 2. Moreover, after careful separation by HPLC (NOVA-PAK silica, 8 dia. × 100 mm, Waters) with n-hexane-ethyl acetate-ethanol (75:25:1), 2-Me crystallized from a solvent mixture of benzene-n-hexane (mp 136–138°C), therefore enabling a structural characterization by a single-crystal X-ray diffraction analysis.

The obtained crystals of 2-Me were in the P21 monoclinic space group with a = 11.007(1), b = 12.881(3), c = 4.890(1)Å, β = 101.98(1)Å and V = 678.3(2)Å³. By using graphite-monochromatized Cu-Kα radiation (λ = 1.5418 Å), 1068 unique reflections (20 ≤ 120°) were measured, 970 of which with |Fo| ≥ 2.67σ(Fo) were considered as having been observed. No absorption corrections were applied, and the structure was solved by a direct method, using SHELXS 86, and the difference Fourier method. The atomic parameters were refined by using the full-matrix least-squares methods with anisotropic temperature factors, while all the hydrogen atoms were located on the difference Fourier map and refined with isotropic temperature factors. Throughout the refinement, function ∑w(|Fo|−|Fc|)² was minimized, with the weighting scheme of 1/w = 1/σ(Fo) during the final refinement stage, to obtain the final R value of 0.057 (Rw = 0.055).³

A computer-generated drawing of the final X-ray model for 2-Me is given in Fig. 2, and the chemical structure of 2 was concluded to be cis-4-(3-acetyl-2,6-dihydroxyphenyl)-2-methoxy-4-butanoide, as shown in Fig. 1. The structure of 1 was deduced to be a trans isomer of 2 (Fig. 1) with respect to the relative configuration at C-2 and C-4, because the spectral data for 1 were very similar to those for 2. Supporting this, NOE was observed in 1-Me between H-2 and H-3α, as well as between H-4 and H-3β, but not between H-2 and H-4. In view of these structures, the compounds appeared to be classified into polyketides, although no biosynthetic study has yet been undertaken. The absolute structures of 1 and 2 were not defined in this study.⁴

A mixture of 1 and 2 inhibited the germination of some phytopathogenic fungi as shown in Table II. The mixture also inhibited the growth of such bacteria as Bacillus subtilis, Escherichia coli and Aeromonas liquefaciens by about 50% at a concentration of 50 ppm.

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References and Notes
2) SHELXS 86, a program for crystal structure solution, was developed by G. M. Sheldrick, University of Göttingen, Germany, 1986.
3) The atomic scattering factors were taken from "International Tables for X-Ray Crystallography."

4) The structures of antafumicins A and B were previously assumed to be 6-acetyl-5-hydroxy-4-methoxy-2-chromanecarboxylic acid, which was presented in the Abstracts of Papers, Autumn Meeting of the Japan Society for Bioscience, Biotechnology, and Agrochemistry, Okinawa, October, 1989, p. 41. The further study by conversion and crystal analysis led us to reexamine the data and propose the revised structures described in this report.