JU-2, a novel phosphorous-containing antifungal antibiotic from *Streptomyces kanamyceticus* M8

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Received 22 September 2000; revised 7 February 2001

A novel phosphorous-containing antifungal antibiotic JU-2 was isolated from *Streptomyces kanamyceticus* M8. Quantitative chemical analysis shows the presence of two phenylalanines, two glucose, one linoleic acid, one erucic acid and one phosphonamide moiety per molecule of the antibiotic. JU-2 shows strong inhibitory activity against various pathogenic and non-pathogenic fungi but no activity against bacteria and yeast.

More than a dozen closely related phosphorous-containing antibacterial antibiotics have been isolated from *Streptomyces* species which possess similar but unusual chemical and biological properties. But there is, however, limited information on phosphorous-containing antifungal antibiotics, viz. phosphinate ester antifungals produced by *Streptomyces hygroscopicus*, phosphazomycins A and C, phospholactomycins produced by *Streptomyces nigrescens* SC-273, and phosphomiside produced by *Streptomyces* species RK-16. In course of studies on the development of high kanamycin-yielding strain of *Streptomyces kanamyceticus* by induced mutation (chemical mutagen NTG), a mutant *S. kanamyceticus* M8 has been isolated which was found to produce a new antibiotic complex active against *Aspergillus niger*. This antibiotic complex was composed of four components and the most active one was found to contain phosphorous other than carbon, hydrogen, oxygen and nitrogen in the molecule. Here we wish to report the isolation and characterization of this active component designated as JU-2.

The organism was grown by shake-flask process at 28°C for 5 days in 500 ml. Erlenmeyer flasks each containing 100 ml of a medium composed of mannitol 2%, NaNO3 0.51%, MgSO4, 7H2O 0.05%, K2HPO4 0.3%, ZnSO4, 7H2O 0.00072%, CaCl2.2H2O 0.004%, FeSO4.7H2O 0.0005% (pH 7.5).

For isolation of the antibiotic the culture broth (20 l.) was first filtered and the filtrate shaken with n-butanol. The butanol layer was separated and then evaporated in vacuum at 45°C to get a dark brown solid mass. This mass was then treated with chloroform. The soluble fraction was evaporated in vacuum to get a light brown solid containing the active substance. This residue was dissolved in chloroform and applied to silica gel preparative thin layer chromatography with ethylacetate-chloroform (4:1). Four separated bands were obtained. The band with Rf 0.33 showing maximum antifungal activity was eluted with chloroform. The active substance was chromatographed by a simple one-dimensional strip technique using different developing solvents, and in all cases, only one single active spot was detected. The eluate was finally concentrated and lyophilized to give 50 mg of JU-2.

JU-2 is a light yellow amorphous substance having no sharp melting point as it decomposes at 150°C ± 2°C. It is soluble in water (neutral, alkaline), butanol, ethanol, chloroform, ethyl acetate and insoluble in ether. It is stable at pHs 4.1-7.5, temperature up to 45°C. It shows positive reactions to KMnO4 test for unsaturation, biuret test for peptide linkage and weakly positive xanthoproteic reaction. It shows negative reactions to FeCl3 test for phenolic hydroxyl group, ninhydrin test for free amino group, Fehling's test for reducing sugar and Elson-Morgan test for amino sugar. Fehling's test for reducing sugar and Elson-Morgan test for amino sugar. JU-2, when acid-hydrated, gives positive reactions to ninhydrin test, Fehling's test and negative to Bial's test for pentose.

On treatment with 0.5N HCl at 60°C for 4 hr followed by neutralization with K2CO3, one equivalent of =-NH2 was determined by ninhydrin reagent. Hydrolysis with 0.5N HCl afforded phosphoric acid as detected by ammonium molybdate perchloric acid reagent. These data suggested the presence of a phosphonamide group in the molecule.

The UV spectrum shows absorption maxima at 225.80, 257.20, 266.80 and 274.80 nm in ethanol. The IR spectrum (KBr) exhibited sharp absorption bands.
Fig. 1 — $^{13}$C NMR Spectrum of JU-2 in CDCl$_3$

Fig. 2 — ESI-MS Spectrum of JU-2
at 700, 1125, 1290, 1385, 1420, 1570, 1720, 2950 and 3450 cm⁻¹. The molecular formula of the compound is C₇₃H₁₁₂N₂O₂₅P. 

$^{13}$C NMR spectrum of 180 p.p.m spectral width (Fig.1) of JU-2 dissolved in CDCl₃ was recorded on Bruker DRX 300, a 300 MHz NMR spectrometer. $^{13}$C NMR suggests the presence of methyl carbons (14.347 ppm), methylene carbons (28.666 ppm), glyceryl carbons (63.755 ppm), olefinic carbons (129.875 ppm). The ESI mass spectrum of JU-2 (Fig 2) was recorded on micromass Quattro II instrument. The sample was dissolved in CH₃CN. Mass spectrum shows the molecular ion peak at m/z 1362 and the molecular weight of the antibiotic can be considered as 1362. Quantitative chemical analysis of the antibiotic shows the presence of two phenylalanine, two glucose, one linoleic acid, one erucic acid and one phosphonamide moiety per molecule.

Judging from all experimental results, the chemical structure of the antibiotic JU-2 (C₇₃H₁₁₂N₂O₂₅P, m.w.1362) has been tentatively suggested as shown in Fig. 3.

These studies indicate that JU-2 is a new phosphorous containing antifungal antibiotic. It shows strong growth inhibitory activity against various pathogenic and non-pathogenic fungi but no activity against bacteria and yeast. It shows strong antifungal activities (0.5 μg/ml MIC) against skin fungi like Trichophyton rubrum and T. mentagrophytes causing superficial mycoses or dematemyoses in animals and humans. The antibiotic is similar in activity to amphotericin B but has greater activity than nystatin against the test organism Aspergillus niger.

The authors acknowledge the financial support to this study by the Department of Biotechnology, Government of India.

References