THE SYNTHESIS OF NITROSOFUNGIN, A NEW ANTIBIOTIC

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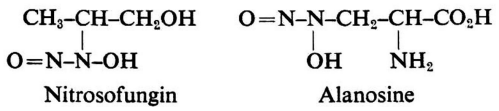
We recently isolated nitrosofungin (a new antifungal antibiotic) from fermentations of a mixed culture of *Streptomyces plicatus* (UC 8272) and a bacterium of the genus Alcaligenes (UC 9152). The fermentation conditions and isolation procedures will be described elsewhere.

Nitrosofungin is 2-*N*-nitrosohydroxylamino-1-propanol, a highly water soluble, acidic compound. It was originally isolated as a mixture of salts. Molecular ions could not be obtained using EI, CI or FAB mass spectral techniques. Crystals suitable for X-ray crystallography could not be obtained and elemental analyses indicated that considerable amounts of inorganic material were present in our best samples.

A study of the spectroscopic data led us to suspect that nitrosofungin was related to alanosine[1], a three carbon antibiotic containing the nitrosohydroxylamino moiety. Hence, a synthesis seemed to be feasible.



Nitrosofungin Alanosine

2-Nitro-1-propanol (Aldrich) was converted into the known crystalline oxalate of 2-hydroxylamino-1-propanol using Pd/C[2]. The free base was prepared as needed by passing a solution of the oxalate over a bed of Dowex 50 X8 (H^+), washing the resin with water, and eluting the hydroxylamine with 1 N NH_4OH. After removal of the ammonia, the oily base was used without further treatment.

The oil obtained from the reduction of 21 g (200 mmol) of 2-nitropropanol was dissolved in 2.0 liters of acetonitrile in a 3-neck round bottom flask equipped with a gas inlet tube, magnetic stirrer and a thermometer. The solution was cooled to 5°C and a stream of dry ammonia was bubbled into the solution. A solution of 25 g (250 mmol) of 1-butyl nitrite in 100 ml of acetonitrile was added dropwise over a three hour period[3]. The ammonia stream was stopped and the solution was decanted from the flask. The ammonium salt of nitrosofungin was collected by washing the precipitate from the wall of the flask with water and lyophilizing the new solution. The gummy ammonium salt was converted to the calcium salt by percolation of an aqueous solution of it over a bed of Amberlite CG 120 (Ca^{++}) (Rohm and Haas cation exchanger). The yield was 12.1 g (43.5 mmol or 43%). It decomposes at 270~275°C.

The calcium salt of synthetic nitrosofungin is identical to the product obtained from fermentation in all respects except optical rotation. It is a free-flowing, white powder, stable in base but not in acid. Hydrogenation with palladium catalyst at low pH affords 2-hydroxylamino-1-propanol. The UV spectrum in 0.01 N NaOH shows a maximum at 245 nm (ε 17650) while in 0.01 N HCl the maximum is at 224 nm (ε 13650). The ^{13}C NMR spectrum (DMSO-d_6, Varian CFT-80, TMS) shows lines at ppm 14.95 (q), 63.13 (t), and 64.51 (d). The ^1H NMR spectrum (DMSO-d_6, Varian XL-200, 30°C, D_2O added) shows a methyl group at δ 1.2 coupled to a methine at 4.25 (J=6.6 Hz). The methine is further coupled to a methylene group at δ 3.6 and 3.4 (J=7.9 and 4.8 Hz). The geminal coupling of the methylene group is 11.2 Hz. The infrared spectrum (reflectance plates) shows strong bands at 3200, 2900, 1580, 1400, 1250, 1175 and 940 cm^{-1}. The elemental analyses are consistent with the molecular formula $C_6H_{14}N_4O_6Ca$. The *pKa* is 5.1.

When 100 μg of this product is loaded onto a 12.7-mm paper disc placed on an agar tray seeded with *Saccharomyces pastorianus* (UC 1342), a 30 mm zone of inhibition is seen after incubation at 28°C for 16 hours. The acute ip LD_{50} is 778 mg/kg in mice.

The success of this synthesis was due largely to the fact that inorganic salts were never introduced, as they are in alternative reduction[4] and nitrosation methods[5]. The fortuitous precipitation of the ammonium salt of nitrosofungin from acetonitrile greatly simplified the final product isolation.

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