Note

3-AMINO-3-DEOXYGLUCOSE PRODUCED BY A *STREPTOMYCES* SP.

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(Received for publication May 26, 1980)

We have discovered that 3-amino-3-deoxyglucose is produced by a new *Streptomyces* species plated out from a Kansas soil. This antibiotic is a constituent of kanamycin[1] which was produced by *Streptomyces kanamyceticus* and of 3-trehalosamine[2] which was produced by *Nocardiopsis trehalosei*. This constitutes the first report of the production of 3-amino-3-deoxyglucose by an organism other than a *Bacillus*[3,4]. This antibiotic was shown to inhibit cell wall synthesis[4]. Two syntheses have been reported[5,6].

The organism was characterized and named *Streptomyces lansus* DIETZ and LI sp. nov. (UC® 8204). L-Diaminopimelic acid was found in the whole cell hydrolysate[7]. This organism displayed many similarities to *Streptomyces venetus*[8] DS 24288 (NRRL 3987, UC® 5789) on Ektachrome[9]. Both are melanin-positive and have spiral spore chains bearing spiny to hairy spores. They are distinguished from one another in their growth on carbon compounds in synthetic medium[10] and by the fact that *S. lansus* grows well at 45°C while *S. venetus* does not grow at 45°C. Antibiotic production and other general cultural and biochemical differences also distinguish the two organisms.

A culture of *S. lansus* on HICKNEY-TRESNER agar was used to inoculate eight 500-ml flasks which contained 100 ml of sterile growth medium each. The medium consisted of 5 g cerelose, 30 ml Brer Rabbit molasses, 10 g sodium glutamate, 2 g ammonium sulfate and 2 g sodium chloride per liter of tap water and was adjusted to pH 7.2 with 5 N sodium hydroxide solution prior to sterilization. The flasks were shaken on a rotary shaker (250 rpm) for three days at 28°C. The contents were used to inoculate 10 liters of the same medium in a fermentation jar. At 28°C the maximum antibiotic concentration was achieved on the 5th day as determined by antibacterial activity on agar trays seeded with *Bacillus subtilis* UC® 564.

Nine liters of beer grown as above was filtered at harvest pH over a pad of Dicalite 4200. The filtrate was adjusted to pH 7 and percolated over a 50×3.8 cm column of granular charcoal. The charcoal bed was washed with deionized water until the eluate was clear. The antibiotic was eluted with 1:1 acetone - water (v/v). The acetone was removed on a rotary evaporator from the active fraction and the aqueous solution was passed over a 50×3.8 cm bed of Dowex 50W×8 (H^+). The resin was washed with deionized water and the antibiotic was eluted with a water to 1.0 M ammonium sulfate gradient. The active fractions were pooled and desalted on a charcoal column as above. Removal of the acetone followed by lyophilization of the aqueous, desalted solution yielded 3.0 g of slightly yellow solid.

The product was reducible with aniline phthalate solution (0.93 g aniline and 1.66 g phthalic acid in 100 ml *n*-butanol saturated with water). The 100 MHz PMR spectrum in D_2O at pD 3 with external TMS showed anomeric doublets at δ 5.45 (J=3.5 cps) and δ 4.90 (J=7.0 cps). This suggested a mixture of anomers of a monosaccharide.[11] The CMR spectrum in D_2O at pD 3 with external TMS showed pairs of peaks as follows: δ 97.4 and 92.6 (D), δ 78.1 and 72.4 (D), δ 72.7 and 69.7 (D), δ 67.9 and 67.6 (D), δ 61.7 and 61.5 (T), δ 59.2 and 56.3 (D). This spectrum was nearly identical to that of an authentic mixture of 3-amino-3-deoxy-α- and β-D-glucose obtained from *Bacillus aminoglucosidicus*[3] kindly provided to us by Dr. B. J. MAGERLEIN of The Upjohn Company.

A sample was derivatized with trimethylsilyl imidazole followed by trifluoroacetic anhydride. The per-O-trimethylsilyl-N-trifluoroacetyl derivative was analyzed on a Hewlett-Packard Model 5992 desktop GC-MS spectrometer. The column (0.4×80 cm) was packed with 3% OV-17 on high efficiency Chromosorb W. Peaks were observed at 9.7 and 10.8 minutes using a thermal gradient

of 3°C/min starting at 140°C. The mass spectra and retention times of these peaks were identical to those obtained for the derivatized, authentic α, β mixture of 3-amino-3-deoxy-D-glucose.

Methanolysis of a second sample followed by derivatization and analysis as described above gave a mixture of the protected methyl α, β-glycosides. The retention times and fragmentation patterns of the major peaks were identical to those obtained for the comparable derivative of the basic sugar from 3-trehalosamine isolated from *Nocardiopsis trehalosei*[2].

Thus, 3-amino-3-deoxy-D-glucose is one of a very few antibiotics that are produced both by a unicellular bacterium (family *Bacillaceae*) and a filamentous bacterium (family *Streptomycetaceae*). It is the only amino sugar antibiotic discovered thus far that is produced by both groups of microorganisms.

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