NOTES

NEOMYCIN B-GLUCOSIDE, A COMPONENT OF MEDIA FERMENTED BY *STREPTOMYCES FRADIAE* 3535

D. PERLMAN and SHEILA K. COWAN

School of Pharmacy, University of Wisconsin, Madison, Wisconsin, U.S.A.

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Recent studies in this laboratory have shown that glycosides are formed when neamine, kanamycin A, or gentamicin C_1 are mixed with aldehydo sugars and solutions incubated at 30°C (or higher) at pH 7.[2] Since these conditions are found in neomycin fermentations it seemed possible that neomycin B-glucoside may occur in neomycin producing fermentations.

Inoculum was prepared by transferring cells from slants of *Streptomyces fradiae* 3535 to tubes of 3% glucose - 3% soybean meal - 1% $CaCO_3$ medium and placed on a rotary shaker at 30°C. After 2-day incubation vegetative growth was transferred to flasks of the same medium which were placed on the rotary shaker. Single flasks (250 ml Erlenmeyer containing 100 ml of medium) were removed from the shaker daily, adjusted to pH 2.0 using 6 N HCl, and shaken vigorously. The acidified mixture was then centrifuged, the pH of the supernatant solution adjusted to pH 7 and analyzed for antibiotic potency (using *Staphylococcus aureus* FDA 209 P with neomycin B sulfate as standard) and identity (using paper ionophoresis at pH 1.9).[1]

Analyses of samples from 2-, 3-, 4- and 5-day fermentations by the ionophoresis procedure showed the presence of small amounts of ninhydrin-positive material which had minimal bioactivity (in bioautographs of the paper ionopherigrams) and the same mobility in the ionopherigrams, *e.g.*, about 80% that of neomycin B, as chemically-prepared neomycin B-glucoside.[2] The pH of the fermentation samples was between pH 7.2 and pH 7.8, and the biopotency of the broth ranged from 60 mcg/ml to 1,720 mcg/ml (using neomycin B sulfate as standard).

The suspected neomycin B-glucoside was isolated from the 2-, 3-, and 4-day fermentations using ion-exchange chromatography on CG-50 (NH_4^+ cycle) and the material compared with material prepared by chemical procedure.[2]

The rotation of the fermentation material (free base) was $+33°[\alpha]_D^{25}$ and that of the chemically produced material was $+35°[\alpha]_D^{25}$ while neomycin B was $+45°[\alpha]_D^{25}$ under the same conditions. Chemical analyses of the free base showed: C, 43.10; H, 7.10; N, 10.1. Calculation for monoglucoside $C_{29}H_{57}O_{18}N_6$: C, 44.79: H, 7.34; N, 10.8.

The biopotency against *Bacillus subtilis*, *Staphylococcus aureus* FDA 209 P, *Escherichia coli* B, *Enterobacter cloacae*, and *Proteus morganii* was between 5% and 33% of the neomycin B sulfate standard. The mobility in the paper ionophoresis system (pH 1.9) and in a tlc system ($CHCl_3$-MeOH-6 N NH_4OH (1 : 3 : 2), v/v) of the material isolated from the fermentations and the chemically-produced material was the same. The half-lives of the two materials at pH 11 at 37°C were about 24 hours, and neomycin B was identified by paper ionophoresis after the alkaline treatment.

When the culture was grown in media containing 6% or 9% glucose, nearly half of the antibiotic produced was found to be the neomycin B-glucoside. We also noted that when glucose was added to 1-, 2-, 3-, 4-, 5- and 6-day fermentation samples to give 8% concentration and the fermentation mixture incubated for an additional 24 hours on the shaker (at 30°C), the biopotency of the samples was reduced by about half, and the paper ionophoresis analysis showed about half of the ninhydrin-positive material in the supernatant was the neomycin B-glucoside.

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References

1) MAEDA, K.; A. YAGI, H. NAGANAWA, S. KONDO & H. UMEZAWA: Application of high-voltage paper electrophoresis for separation

and identification of antibiotics. J. Antibiotics 22: 635~636, 1969

2) PERLMAN, D.; T. ENDŌ, R.S. HINZ, S.K. COWAN & S. ENDŌ: Properties of glycosides of neamine, kanamycin A, and gentamicin C_1. J. Antibiotics 27: 525~528, 1974