

ANTIMETABOLITES PRODUCED  
BY MICROORGANISMS. III<sup>1,2)</sup>  
2-AMINOPURINE-6-THIOL  
(THIOGUANINE)

Sir:

In the course of investigations into antimetabolites produced by microorganisms,<sup>1,2)</sup> we detected a substance in the fermentation broth of an unidentified pseudomonad\* that inhibited the growth of *Escherichia coli* in a minimal agar medium<sup>3)</sup>. This inhibition did not occur when either adenine or guanine was present in the assay medium. Antimetabolite production was carried out in a medium containing (in g/liter): glucose, 10; yeast extract (Difco), 2; asparagine, 0.5; and  $K_2HPO_4$ , 0.5. The pH was maintained at  $6.6 \pm 0.2$  by the addition of 5 N NaOH. The fermentation, carried out at 28°C in an aerated stirred fermentor, was harvested at 32 hours.

The active substance was adsorbed from 350 gal. (ca. 1,325 liters) of filtered broth (antimetabolite concentration, 4 mg/liter) onto a 50-liter column of Dowex 50WX4 resin, 50~100 mesh in the  $H^+$  form and eluted with 10% aqueous pyridine solution. After concentration of the eluate at reduced pressure the antimetabolite was adsorbed onto 30 g Norite A mixed with 30 g Celite analytical filter aid. The charcoal was washed with pyridine and the activity was eluted with 4 liters benzene-methanol (1:1, v/v). The eluate was evaporated at reduced pressure to give a 3 g residue which was triturated with 100 ml 0.1 N HCl. The insoluble residue, 600 mg, was separated by filtration and then dissolved in 250 ml boiling methanol-0.1 M aqueous solution of HCl (1:1, v/v). During evaporation at reduced pressure to a small volume (25 ml), 380 mg of crystalline material separated. After two recrystallizations from methanol-0.1 M aqueous HCl (4:1, v/v), the material was converted to the base by addition of 200  $\mu$ l pyridine to a solution of 220 mg hydrochloride salt in 40 ml boiling water. After recrystallization from 150 ml water,

light yellow needles were obtained: Anal. calcd for  $C_5H_5N_5S$ : C 35.92, H 3.01, N 41.89, S 19.18. Found: C 35.69, H 3.03, N 41.85, S 18.31, 18.01. The infrared and ultraviolet spectra of the substance were identical to those of synthetic 2-aminopurine-6-thiol (thioguanine)<sup>4)</sup>, an antileukemia drug. This is the first report of the natural occurrence of this compound.

Since purine analogs usually occur in nature as nucleosides and since the deoxynucleoside in particular would not have survived the cation-exchange step in the purification procedure, an alternative milder procedure was devised to determine whether either of the nucleosides were present in the broth. A small scale fermentation broth was filtered and treated with charcoal as described above. Solids obtained by evaporation of the benzene-methanol eluate were then chromatographed on a Bio-Rad cellulose MX column developed with water. Active fractions were then subjected to thin-layer chromatography using several solvent systems<sup>5)</sup>. Neither of the nucleosides was present within the limits of detection by bioautography and ultraviolet light absorption. A control experiment proved the nucleosides would have been recovered by this procedure.

Acknowledgements

We are indebted to members of the Fermentation Pilot Plant under Mr. B. TABENKIN and the Physical Chemistry Department under Dr. P. BOMMER for their contributions to the work.

JAMES P. SCANNELL  
DAVID L. PRUESS  
MARTHA KELLETT  
THOMAS C. DEMNY  
ARTHUR STEMPER

Chemical Research Department  
Hoffmann-La Roche Inc.  
Nutley, New Jersey 07110, U.S.A.

(Received February 16, 1971)

\* *Pseudomonas* sp. GH. (HLR 186B) kindly supplied by Prof. J. B. NEILANDS, Department of Biochemistry, University of California, Berkeley, Calif., U.S.A.

## References

- 1) SCANNELL, J. P.; D. L. PRUESS, T. C. DEMNY, T. WILLIAMS & A. STEMPEL: L-3-(2,5-Dihydrophenyl)alanine, an antimetabolite of L-phenylalanine produced by a *Streptomyces*. J. Antibiotics 23 : 618~619, 1970
- 2) SCANNELL, J. P.; D. L. PRUESS, T. C. DEMNY, F. WEISS, T. WILLIAMS & A. STEMPEL: L-2-Amino-4-pentynoic acid: antimetabolites from microorganisms. II. J. Antibiotics 24 : 219~224, 1971
- 3) DAVIS, B. D. & C. S. MINGIOLI: Mutants of *Escherichia coli* requiring methionine or B<sub>12</sub>. J. Bact. 60 : 17~23, 1950
- 4) ELION, G. B. & G. H. HITCHINGS: The synthesis of 6-thioguanine. J. Am. Chem. Soc. 77 : 1676~1677, 1955
- 5) SCANNELL, J.P. & G.H. HITCHINGS: Thioguanine in deoxyribonucleic acid from tumors of 6-mercaptopurine-treated mice. Proc. Soc. Exp. Biol. & Med. 122 : 627~629, 1966