EFFECT OF STRUCTURE ON NUCLEOSIDE ANTAGONIST ACTIVITY[1]

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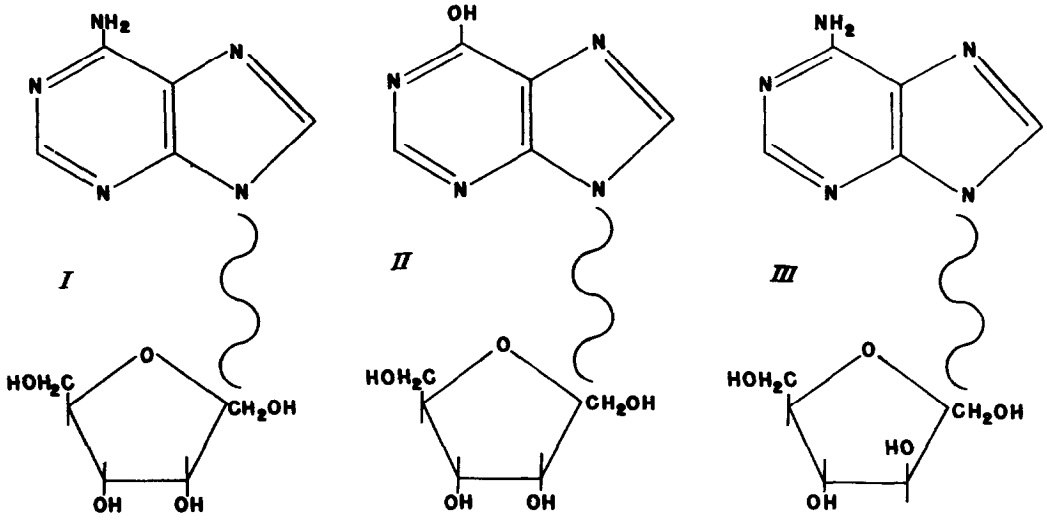
Psicofuranine[2], (I), a nucleoside antagonist, inhibits xanthosine-5'-phosphate aminase (Hanka, 1960; Slechta, 1960); it is also bacteriostatic to Escherichia coli at 9μg/ml and acts as a derepressor for XMP aminase (Moyed, 1961). This communication describes the chemical conversion of psicofuranine (6-amino-9-D-psicofuranosylpurine) (I) to the new compound 6-hydroxy-9-D-psicofuranosylpurine (II) by diazotization (see, Shuster and Kaplan, 1953). Compound II is not bacteriostatic against E. coli at 500μg/ml or less, but does enhance the inhibitory action of psicofuranine (Fig. 1, curve 4).



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[2] Generously supplied by Dr. G. M. Savage, The Upjohn Company, Kalamazoo, Michigan.

Inosine, hypoxanthine and xanthosine exhibit the same effect as (II) (curve 4). Structural requirements for the bacteriostatic action of psicofuranine are further explained in the present work.

Psicofuranine concentration required to retard growth depends on the organism; in E. coli this is 10 to 25μg/ml, in A. aerogenes 50 to 75μg/ml, while P. aeruginosa is not inhibited by > 200μg/ml.

Elementary analysis of II - m.p., 166.5-168° d.; formula $C_{11}H_{14}N_4O_6$[3];

calculated: C 44.30, H 4.70, N 18.79, O 32.21

found: C 44.70, H 4.95, N 18.82, O 31.52

Addition of analog II, or any of the naturally occurring hydroxypurines, gave the same effect as a five fold increase in psicofuranine concentration. Fiveμg/ml of psicofuranine I has no bacteriostatic effect, Figure 1; IMP dehydrogenase and XMP aminase activity increase at this concentration (Moyed, 1961). However, upon the addition of any of the hydroxypurines to a tube containing 5μg/ml I resulted in some growth inhibition (curve 3).

Compounds I and III[4] differ only in the stereochemical configuration of the hydroxy group at C-3 of the ketohexose (D-psicose vs. D-fructose), thus the contribution of the sugar moiety to bacteriostatic activity was determined.

Use of compound III (50μg/ml) instead of psicofuranine gave no growth inhibition; use of III with I gave the same inhibition as I, (curve 2). Thus, the ketohexose, psicose and the amino group of the purine moiety are essential for bacteriostatic activity. Combining II and III did not produce a synergistic effect on growth inhibition.

Compound I prevents the synthesis of GMP by blocking XMP aminase and addition of guanine reverses inhibition; the synergistic effect observed by combining psicofuranine with compound II, inosine, hypoxanthine or xanthosine is also reversed by guanine. Reversal of the synergism suggests an effect

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[3] Microanalyses by Galbraith Laboratories, Knoxville, Tennessee.

[4] Compound III (both α and β forms) was kindly supplied by Dr. E. J. Reist of Stanford Research Institute, Menlo Park, California.

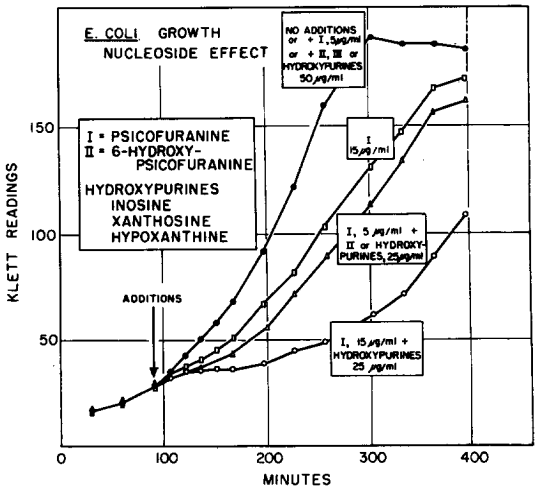

Figure 1, Growth of E. coli: Effect of nucleosides and medium as per Moyed (1961) minimal, 37°, aerated, culture diluted 1:25 at 0 time.

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of the hydroxy purines at a point in the nucleotide interconversion cycle (Magasanik and Karibian, 1960). The possibility of I, in combination with the hydroxy purines, inhibiting the same enzyme (XMP aminase) as I alone has been studied with partially purified (E. coli) XMP aminase, and no increased inhibition found.

From our results, it appears that combinations of normal metabolic intermediates (inosine, hypoxanthine and xanthosine) with a nucleoside antagonist I enhances the bacteriostatic effect of the original antagonist. This synergism may be attributed to the inhibition of more than one enzyme. The true implication of the reversal of this synergism by guanine is not clear at present.

The data presented show the necessity for the amino group on C-6 of the purine moiety of I for bacteriostatic activity as well as a requirement for a specific stereochemical arrangement of the hydroxy group at C-3 of the ketohexose. Alteration of either the amino group or the ketohexose completely destroys the bacteriostatic activity.

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