2H-1,3-OXAZINE-2,6(3H)-DIONE, A NEW PYRIMIDINE ANTIMETABOLITE

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SUMMARY

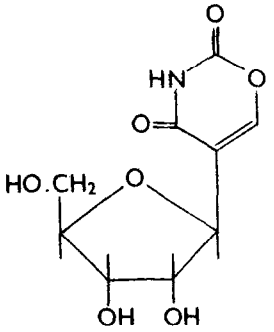
2H-1,3-Oxazine-2,6(3H)-dione inhibits the growth of Escherichia coli B, the inhibition being complete at a concentration of 10^{-4}M. It may be relieved with uridine, cytidine and partly with uracil. Orotic acid, cytosine, purine bases and purine ribonucleosides show no effect. At a molar ratio of uridine to the inhibitor of 1:2 the inhibition is completely suppressed. 2H-1,3-Oxazine-2,6(3H)-dione is thus a novel inhibitor of the biosynthesis of pyrimidine precursors of nucleic acids.

INTRODUCTION

Search for new antimetabolites of nucleic acid components is stimulated not only by scientific endeavor but also by practical aspects. A number of compounds of this category found important application in human medicine (e.g., 6-mercaptopurine, imuran, derivatives of 5-fluorouracil and 6-azauridine).

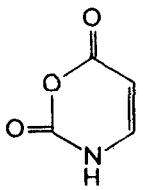
The majority of antimetabolites of nucleic acid components were discovered during more or less empirical synthetic attempts, the modification of the natural components being thus decided upon on the basis of experimental intuition. The discovery of the present antimetabolite has a somewhat different history. Recently Japanese authors described an antibiotic designated as oxazinomycin (1). The antibiotic was isolated also by another Japanese group and was called minimycin (2). In the last-mentioned team (3) the structure of the antibiotic was determined as 5-β-D-ribofuranosyl-2H-1,3-oxazine-2,4(3H)-dione (formula

I) where the heterocyclic moiety is attached to ribofuranose by a C-C bond.



(I)

The occurrence of a 1,3-oxazine ring in a natural compound led us to the idea of checking the biological activity of 2H-1,3-oxazine--2,6(3H)-dione (formula II) which differs from the aglycone of oxazinomycin (minimycin) in the position of the NH group. By placing the NH group in position 1 we were thus able to produce a model compound of the type of "3-oxauracil".



(II)

MATERIAL AND METHODS

2H-1,3-Oxazine-2,6(3H)-dione was prepared according to Washburn et al. (4). After recrystallization from ethyl acetate a preparation was obtained which melted at 148-153°C, and showed a λ_{max} (H_2O) at 264 nm (log ε 3.90).

Escherichia coli B was grown in the following medium: 20 mM glucose, 0.1 M phosphate buffer of pH 7.4, 20 mM $(NH_4)_2SO_4$, 10 mM NaCl, 2 mM $MgSO_4$, 0.05 mM $CaCl_2$ and 0.01 mM $FeSO_4$. Cultivation took place under stationary conditions at 37°C and growth was followed by measuring absorbance at 575 nm.

TABLE I

Antagonism between 2H-1,3-oxazine-2,6(3H)-dione and nucleic acid precursors

Growth in the absence of inhibitors and precursors is taken as 100 %. Concentration of inhibitor and precursors was 10^{-4}M.

Precursor	Growth (%)
Orotic acid	0
Uracil	64
Cytosine	0
Adenine	0
Guanine	0
Uridine	100
Cytidine	100
Adenosine	0
Guanosine	0

RESULTS AND DISCUSSION

It was found that 2H-1,3-oxazine-2,6(3H)-dione in a mineral medium with glucose displays a relatively powerful inhibitory effect on the growth of Escherichia coli B. At a concentration of 10^{-4}M the inhibition was complete. In another experimental series the mode of inhibitory action of 2H-1,3-oxazine-2,6(3H)-dione was examined. Table I shows that the growth-inhibiting effect can be completely relieved with uridine and cytidine, partly with uracil. Orotic acid, cytosine and purine bases or purine ribonucleosides did not suppress the inhibition.

It follows from the results summarized in Table II that the inhibitory effect of 2H-1,3-oxazine-2,6(3H)-dione is completely removed

by uridine at one-half molar concentration of the inhibitor.

The above findings permit to conclude that 2H-1,3-oxazine--2,6(3H)-dione interferes specifically with the de novo biosynthesis of the pyrimidine precursors without impairing the utilization of preformed pyrimidines. A detailed study of the mode of action of the new antipyrimidine, together with an investigation of its virostatic and cancerostatic effects, is now under way.

TABLE II

Effect of different concentrations of uridine on the inhibition of Escherichia coli B growth by 2H-1,3-oxazine-2,6(3H)-dione

Concentration of 2H-1,3-oxazine-2,6(3H)-dione	Concentration of uridine	Growth (%)
0	0	100
10^{-4}M	0	0
10^{-4}M	10^{-6}M	0
10^{-4}M	5 x 10^{-6}M	26
10^{-4}M	10^{-5}M	50
10^{-4}M	5 x 10^{-5}M	105

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