Tetrazole Analogs of Various Amino Acids as Possible Bacterial Antagonists

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RECENTLY, the synthesis of the tetrazole analogs of several amino acids was described by McManus and Herbst (1). Since we had an opportunity to obtain several of these compounds,[1] it was of interest to determine whether they had any activity as amino acid antagonists in bacteria.

The tetrazole analogs tested were as follows: I, 5-aminomethyltetrazole (glycine analog); II, 5-(1′-aminoethyl)tetrazole (DL-alanine analog); III, 5-(2′-aminoethyl)tetrazole (β-alanine analog); IV, 5-(1′-aminopropyl)tetrazole (DL-α-aminobutyric acid analog); and V, 5-(1′-amino-2′-phenylethyl)tetrazole (DL-phenylalanine analog).

Each of the compounds was tested with a microorganism which synthesizes its own amino acids, and certain compounds with microorganisms which require specific amino acids for growth. All compounds were tested for their ability to inhibit the growth of *Escherichia coli* W in the synthetic medium of Davis and Mingioli (2).

In addition, test compounds I and V were tested for their ability to inhibit utilization of glycine and phenylalanine in the amino acid basal medium of Barton-Wright (3) using *Leuconostoc mesenteroides* P60, an organism requiring these amino acids. Compound II was also tested for antagonism of DL-alanine utilization in the medium of Sauberlich and Baumann (4) using *Pediococcus cerevisiae* (ATCC No. 8081), an alanine-dependent organism. Neutralized solutions of test compounds, amino acids, and basal media were sterilized by Seitz filtration and portions added to previously autoclaved culture tubes containing graded levels of sterile, distilled water. Only freshly prepared solutions of the test compounds were used. Final assay volumes were 6 ml. per culture tube (18 × 150 mm.). In the preparation of the inocula, precautions were taken to minimize the carry-over of preformed amino acids by appropriate washing of the cultures and by the use of small inocula. Incubation periods of 24 hours were used with *E. coli* and *L. mesenteroides*, and 72 hours with *P. cerevisiae*. An incubation temperature of 35° was used with all cultures. Growth was measured turbidimetrically as absorbance using a Coleman junior spectrophotometer at a wavelength of 620 mμ.

All of the tetrazole analogs studied, at a final concentration of 1 mg. of test compound per ml. (molar range of 0.005 to 0.010), failed to inhibit the growth of *E. coli* in a 24-hour assay. This clearly indicates that these compounds (I through V) are not potent inhibitors of the synthesis and/or utilization of amino acids.

TABLE I.—ANTAGONISM OF DL-ALANINE UTILIZATION BY 5-(1′-AMINOETHYL)TETRAZOLE IN *P. cerevisiae*

Molarity 5-(1′-Aminoethyl)-tetrazole	Molarity DL-Alanine	Molar Ratio Antagonist to Metabolite	% Inhibition of Growth, 72 hours at 35°
0.022	0.000093	237/1	98
0.022	0.000186	118/1	38
0.022	0.000372	59/1	19
0.040	0.000093	430/1	99
0.040	0.000186	215/1	50
0.040	0.000372	108/1	10
0.040	0.000744	54/1	4
0.040	0.001488	27/1	3

Compound I at a 0.04 *M* concentration when tested with *L. mesenteroides* in the presence of 0.0004 *M* glycine (half maximal growth) inhibited the growth of *L. mesenteroides* by 28%. No inhibition of growth was observed at 0.005 *M*, 0.01 *M*, and 0.02 *M* concentrations. This low order of activity was not sufficient to warrant further studies.

In the presence of 0.0002 *M* DL-phenylalanine, the level required for half maximal growth, no inhibition of growth was observed at 0.05 *M* and 0.01 *M* concentrations of compound V. It was not possible to test higher concentrations due to the low solubility of this compound at a neutral pH.

5-(1′-Aminoethyl)tetrazole (compound II) was shown to be an antagonist of DL-alanine utilization with *P. cerevisiae*. This anatagonism was readily reversed by DL-alanine. The compound must be considered a weak inhibitor, however, in that approximately a 200–400 to 1 molar ratio of antagonist to metabolite was needed to obtain a 50 to 99% inhibition of growth (Table I).

Further tests on compounds I, II, and V with *L. mesenteroides* and *P. cervisiae* which require glycine, phenylalanine, or α-alanine, respectively, showed that the analogs did not support growth in place of the respective amino acids.

In summary, tetrazole analogs of five different amino acids were found to have little or no activity as inhibitors of bacterial growth in chemically defined media. As yet, none of the available tetrazole analogs have been studied in isolated enzyme systems.

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