Alanosine is an extracellular product of *Streptomyces alanosinus* which has been shown to have antibiotic, antitumor and immunosuppressive activity [1-3]. The structure of the natural product is L-(+)-2-amino-3-(hydroxy-nitrosamino) propionic acid [4]. Studies of its mode of action suggest that it can inhibit both AMP and pyrimidine biosynthesis in microbes [5], and it was suggested that the drug affected both adenylosuccinate synthetase and aspartase transcarbamylase. Recently, Graff and Plagemann [6] have reported that in Novikoff hepatoma cells the inhibition is specifically for adenylosuccinate synthetase, by alanosine [6]. Gale and Smith [9] have suggested that a metabolite of alanosine was responsible for the inhibition observed which may be what occurs in the studies of Graff and Plagemann [6].

To further evaluate the roles of alanosine and hadacidin, the inhibition toward *E. coli* adenylosuccinate synthetase, aspartase, asparaginase and aspartate transcarbamylase was studied. These enzymes all have aspartate as a substrate or product allowing conclusions to be drawn about the specificity of aspartate binding sites in the different proteins. Also, the ability of a mammalian adenylosuccinate synthetase to utilize alanosine as a substrate was tested.

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Hadasidin (N-formyl hydroxyiminoacetic acid) is an antibiotic from *Penicillium frequemcents* which also inhibits adenylosuccinate synthetase [7], causing a decrease in AMP biosynthesis without inhibition of GMP formation.

The inhibition of adenylosuccinate synthetase is competitive with the natural substrate aspartate [8].

Gale and Smith [9] have compared the effect of alanosine and hadacidin on partially purified *Escherichia coli* adenylosuccinate synthetase. Hadacidin was a potent inhibitor competitive with aspartate as previously determined, but alanosine did not inhibit the enzyme. The similarity of the structure of alanosine to aspartate made this result somewhat unexpected, particularly in view of the more recently discovered specific inhibition in vivo of adenylosuccinate synthetase by alanosine [6]. Gale and Smith [9] have suggested that a metabolite of alanosine was responsible for the inhibition observed which may be what occurs in the studies of Graff and Plagemann [6].

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routinely performed as for the adenylosuccinate synthetase step, the effect of the inhibitors on aspartase so inhibition, if any, should be observed. The enzymes studied at concentrations 1000-fold higher than its $K_{i}$ for the synthetase. This suggests a multiplicity of binding sites for aspartate and may allow future synthesis of very specific antimetabolites.

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REFERENCES


Conjugation of hydroxyphenylhydantoin and hydroxyphenobarbital in rat liver microsomes. Induction by phenobarbital*

(Received 30 April 1976; accepted 7 January 1977)

Phenobarbital (PB) and diphenyldantoin (DPH) are metabolized through hydroxylation [1, 2] followed by conjugation of 80% and 60-70% of the total drug, respectively, with UDP glucuronic acid (UDPGA). The hydroxylation has been studied in detail [3, 4] but the conjugation of the hydroxylated metabolites with UDPGA has never been examined in detail.

A suitable radiochemical procedure for determining the activity of UDP glucuronyltransferase (UDPGT (E.C. 2.4.1.17) unspecified acceptor) versus these metabolites was required and previously developed in our laboratory [5].

We studied the activity of rat liver UDPGT towards both metabolites and in addition the effect of phenobarbital pretreatment of rats was examined.

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