USE OF ^{19}F NMR FOR THE INTERACTION OF FLUORO KETOACIDS AND FLUORO AMINO-ACIDS WITH ESCHERICHIA COLI ASPARTATE TRANSAMINASE

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Monofluoro-oxaloacetate (FOA) was prepared from condensation of ethyl monofluoroacetate with diethyl oxalate, then hydrolysis of the ethyl ester. Difluoro-oxaloacetate (F_2OA) was prepared by fluorination with ClO_3F on ditertiobutyl oxaloacetate and hydrolysis of the tertiobutyl ester.

Monofluoro-oxaloacetate, Difluoro-oxaloacetate are good competitive inhibitors of 2-oxo glutarate of E-Coli Aspartate Transaminase. pF phenylalanine, mF phenylalanine, Pentafluorophenylalanine are moderate competitive inhibitors and in a small amount substrates of this enzyme. The effector properties of monofluoroaspartate and difluoroaspartate are in progress.

Reactivity of monofluoro oxaloacetate versus PMP, the coenzyme in its aminic form of the enzyme can be tested by ^{19}F NMR spectroscopy. The evolution af the ^{19}F N.M.R. spectra at pH $\sim$ 9.5 was described. Three doublets are observed (triplets in D_2O) : a sharp one, two others with appearance of not resolved fine structure, one of which identified to fluoroaspartate. At pH $\sim$ 5 only one doublet is observed (in D_2O a triplet slowly appears when doublet disappears). The fine structures, the pH chemical shift variations (^{19}F and ^{1}H N M R) suggest that the three doublets are the fluoro ketimine, aldimine and amino acid products of reaction with PMP. The rate of reaction ketimine to aldimine is constante from 9 to 10.5 but is smaller at pH 8.

Enolization and deuteration does not occur on FOA contrarly to the case of non substituted oxaloacetate.

These reactions are important to understand the enzymatic reaction in the catalytic site of the enzyme and to design more specific inhibitors.

Reversible association of the fluorinated effectors with the enzyme is studied by ^{19}F NMR, from solutions with variable ratio of effector:enzyme. The two parameters for this association, equilibrium constant and chemical shift of the effector in the enzyme site are obtained and are compared with the corresponding values for the reaction of the fluorinated effectors with the coenzyme.