On the reaction of hydroxylamine with esters of amino acids

It is well known that under alkaline conditions hydroxylamine reacts with esters of carboxylic acids to give hydroxamic acids. This reaction has long been used for the quantitative determination of esters by estimating the resulting hydroxamic acid by means of the color reaction with $FeCl_3$[1-6]. Under neutral conditions, however, normally only "activated" esters and acid anhydrides react with hydroxylamine, a fact that has been taken advantage of in the determination of anhydrides in the presence of esters and in the testing for "activated" carboxyl groups in general[6,7].

Esters of amino acids are generally more labile than ordinary carboxylic acid esters. In view of the importance that the formation of hydroxamic acids under neutral or slightly alkaline conditions has recently assumed in the search for activated amino acids in the process of protein synthesis[8-10], it seemed of interest to determine the rate of reaction of simple amino acid esters with hydroxylamine under the conditions normally employed in the above-mentioned studies.

Leucylmethylester was used as a model substance. It was dissolved in dlstilled water at a concentration of 4 μmoles/ml. Aliquots of this solution were then added to equal volumes of 2 M hydroxylamine solutions at different pH's and incubated at 37°. Samples of 3 ml each were withdrawn at intervals and pipetted into 1.4 ml 100% (w/v) trichloracetic acid (TCA) adjusted to pH 0.8 (as read with a glass electrode) with 25 N NaOH.

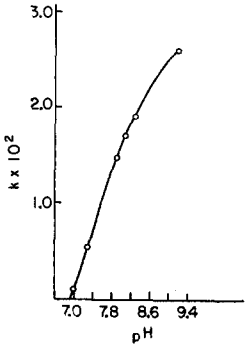

Fig. 1.

The amount of hydroxamic acid formed was determined by the method of SCHWEET[11], using a standard of leucine hydroxamic acid. The slopes of the standard hydroxamic acid curves vary in a linear fashion with the final pH of the reaction mixture. The slope (extinction for 1 μmole leucine hydroxamic acid per sample) at pH 0.4 was 0.111, and increased by 0.027 for every tenth of a pH unit up to pH 0.9.

It was found that at pH's between 7 and 9, reactions of the ester with the hydroxylamine proceeded at reasonable rates as can be seen from Fig. 1, which shows a plot of the rate constants as a function of pH. At all pH's, the reaction is initially first order with respect to the ester, but only in the narrow pH range of 7.6 to 8.0 did the reaction proceed to completion without complications. At pH's below and above this range the reaction does not proceed to completion due to instability of the ester and/or the hydroxamic acid formed. At pH's above 9 the hydrolysis of the hydroxamic acid proceeds so rapidly as to preclude a useful kinetic investigation under the present conditions.

Preliminary experiments have indicated that there is considerable variation in the rate of reaction of esters of amino acids with hydroxylamine depending on the nature of both the alcohol and the amino acid, so that not all esters of amino acids need necessarily interfere with the hydroxamic acid assay for active acyl groups. The data reported here do point out, however, that the activated amino acid "trapped" with hydroxylamine could be in something other than an anhydride linkage. The 3'-valyl ester of adenylic acid obtained by WIELAND AND PFLEIDERER[12] and the ribose-acetylated adenylic acid obtained by JENCKS[13], both of which react very rapidly with hydroxylamine under neutral conditions, illustrate the point further. It would also be reasonable to suppose that the active leucyl residue which has been found by HOAGLAND AND ZAMECNIK[14] to be bound to an RNA fraction prior to its incorporation into liver microsomal particles might be esterifying the 2' position of the ribose.

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