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NOTE

Effect of Temperature, pH, Hydrindantin, Ascorbic Acid and Organic Solvents in the Colorimetric Estimation of α-Amino Acids with Ninhydrin

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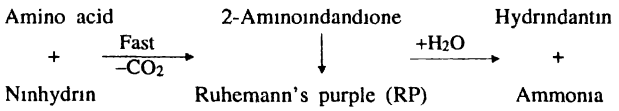
The interaction of ninhydrin with amino acid is well-known. A number of modifications in the ninhydrin reagents have been made with the help of hydrindatin[1], reducing agents[2] and organic solvents[3] for a reproducible and better result. Speckman *et al.*[4] reported that the presence of reduced form of ninhydrin (hydrindantin) is essential for the colorimetric estimation of amino acids. The drawback with this reagent is its instability in presence of light and air. Therefore, it must be stored and manipulated in dark under nitrogen atmosphere. However, there is no report on the individual effect of temperature, pH, hydrindantin, reducing agents and organic solvents in the development of colour, and therefore the effects of temperature, pH, hydrindantin and ascorbic acid are reported here.

Results and Discussion

Reaction of α-amino acids with ninhydrin gives carbon dioxide, Ruhemann's purple (RP), ammonia and hydrindantin as products. In this reaction 2-aminoindandione is a stable intermediate[5], whose formation is much faster in comparison to its reaction with ninhydrin. Therefore, the overall reaction can be represented as



The evolution of CO_2 was completed in 10 min at 80°, while the colour formation was negligible within this time interval.

The organic solvents (DMSO and methyl cellosolve) increased the intensity of RP during the interaction of ninhydrin with α-amino acids. Hydrindantin formed alongwith RP, was highly insoluble in water and required the presence of water-miscible oragnic solvent. Oxygen played an important role in the formation of RP. In presence of atmoshpheric oxygen, RP was not formed. This indicates that there is an intermediate formed during the interaction of α-amino acids with ninhydrin which is highly sensitive towards oxygen.

If the ninhydrin solution was prepared in presence of modified reagent, it was necessary to explain the effect of each component. Hydrindantin, ascorbic acid and organic solvents only enhanced the intensity of RP and did not affect the mechanism of the reaction. Out of these, temperature and oxygen are the important parameters which govern the formation of RP.

The effect of temperature on the formation of RP was studied at 50–95° at pH 5.0. At temperature below 70°, the formation of RP was negligible, but above it the absorbance of RP was found to increase with the increase in temperature. The maximum colour yield was observed at 95°. The intensity of colour increased with the increase in pH and thereafter decreased sharply on further increase in pH from 6.0 to 9.0. Thus the optimum pH was found in the vicinity of 5.0–6.0. The intensity of colour was found to increase with addition of hydrindantin. The enhancement in the intensity is, firstly, due to decomposition of hydrindantin into 2-hydroxyindandione through retro-aldol cleavage. Hydrindantin acts as a source of 2-hydroxyindan-

dione, which is an intermediate in the formation of RP. But in presence of O_2, hydrindantin becomes the source of ninhydrin due to the aerial oxidation[5] of protonated 2-hydroxyindandione. Secondly, RP and hydrindantin are formed through parallel reactions. Addition of hydrindantin (one of the products) to the reaction mixture shifts the equilibrium in favour of RP.

The absorbance of the RP increased with the addition of ascorbic acid (2×10^{-3} mol dm^{-3}). On further increase in concentration of ascorbic acid to 12.0×10^{-3} mol dm^{-3} the RP was not formed and the reaction mixture turned red instead due to the formation of hydrindantin. The reduction of ninhydrin with ascorbic acid is very fast in comparison to its reaction with α-amino acids. Therefore the role of ascorbic acid is only to reduce ninhydrin to hydrindantin.

Experimental

The amino acids gly., α-ala., phe., ser., aspr., asp., his., (all B.D.H.) and ninhydrin (Sisco) were used as such. The DMSO and methyl cellosolve (modified agents) were studied on the formation of RP only.

Solutions of the amino acids, ninhydrin, hydrindantin, DMSO and methyl cellosolve were prepared in acetic acid-sodium acetate buffer at different pH. The pH measurements were made by using an Elico digital pH meter.

Methods : The reaction mixture (10 ml) containing 0.2 mol dm^{-3} ninhydrin solution (1 ml) and amino acid solution (0.01 ml) was taken in a reaction vessel fitted with a double surface condenser to minimise evaporation and kept in a constant temperature oil bath. Pure N_2 was bubbled through the reaction mixture. The spectrum of the colour developed was measured (Elico CL-27 digital spectrophotometer) against reagent blank constituting ninhydrin solution (0.5 ml) and buffer solution (4.5 ml) simultaneously.

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