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Microdetermination of Hexosamines

The practical methods for the determination of hexosamines were those of Elson–Morgan,[1] Morgan–Elson,[2] and Dische–Borenfreund.[3] Recently, two new spectrophotometric methods for amino sugars were reported; one involves the reaction with trinitrobenzene-1-sulfonic acid[4] and the other employs the color reaction with *p*-nitrobenzenediazonium salt in alkaline medium.[5] Previously, a specific procedure using *p*-nitrobenzaldehyde was devised in our laboratory.[6] The method described in the present paper utilizes nitrous acid deamination of hexosamines in the first stage of the colorimetry as in Dische–Borenfreund method, but color development from the deaminated hexosamine is based on a completely different principle from that of Dische–Borenfreund procedure. 3-Methyl-2-benzothiazolone hydrazone[7] (III) reacted readily with 2,5-anhydrohexoses (II) produced by the deamination of hexosamines (I) and exhibited a blue color by addition of ferric chloride solution. A possible mechanism of this color reaction is shown in Chart 1. By the use of glucosamine and galactosamine the determination of hexosamines was examined and high sensitivity down to 1 μg/ml and excellent selectivity were obtained.

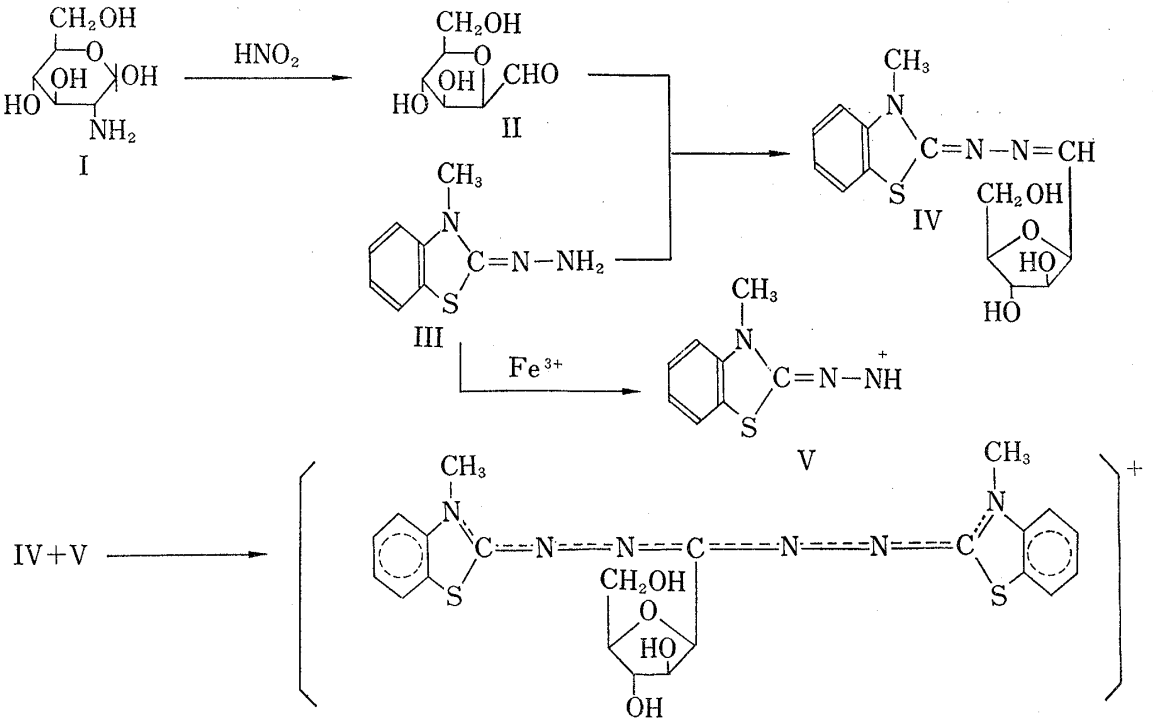

Chart 1. Probable Mechanism of Color Reaction

Microdetermination of hexosamines: To 1 ml of the aqueous test solution containing hexosamines each 1 ml of 5% $NaNO_2$ solution and 5% $KHSO_4$ solution is added, mixed

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2) W.J.T. Morgan and L.A. Elson, *Biochem. J.*, **28**, 988 (1934).
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thoroughly, and allowed to stand for 15 min. To the mixture is added 1 ml of 12.5% ammonium sulfamate solution and the mixture is allowed to stand with occasional shaking for 5 min. To the solution is then added 1 ml of 0.5% 3-methyl-2-benzothiazolone hydrazone hydrochloride solution and the mixture is left standing for 60 min. Finally, 1 ml of 0.5% $FeCl_3$ is added and the absorbance of the resulting blue color is measured at 650 mμ against the reagent blank after 30 min. The absorbance is proportional to the concentration of hexosamines in the range of 1—30 μg/ml of the test solution.

When applied to chondroitin sulfate A and C, hexosamine value obtained by the present method (32.8% and 32.0%, respectively) showed good agreement with that expected (33.9% for both mucopolysaccharide) from nitrogen content of each polysaccharide. In addition, only 2 hr of hydrolysis in 2 N HCl at 100° was required whereas Elson–Morgan method or Dische–Borenfreund method requires 16 hr of hydrolysis under the same conditions.

Details of the experiment will be reported in the near future.

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