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 acid⁴⁾ 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 hydrazone⁷⁾ (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 $\mu\text{g/ml}$ 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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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 $\text{m}\mu$ against the reagent blank after 30 min. The absorbance is proportional to the concentration of hexosamines in the range of 1–30 $\mu\text{g}/\text{ml}$ 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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