Note

Acid Degradation of Amino-monosaccharides and Amino-polysaccharides

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Received February 25, 1988

Quantitative analysis by gas-liquid chromatography (GLC) is an important method for determining the chemical compositions of complex polysaccharides. In order to avoid the formation of anomeric mixtures, it involves^{1,2)} the total acid hydrolysis of the parent polysaccharide into its constituent monosaccharides, and subsequent borohydride-reduction and peracetylation into alditol acetates. In the course of our investigations of chitinous polysaccharides, the need arose for a quantitative assay of glucosamine, and we reported³⁾ a problem in the quantitative analysis of glucosamine by GLC. This prompted us to investigate suitable conditions for quantitatively analyzing glucosamine as glucosaminitol acetate by GLC.

GLC analysis of amino-alditol acetates is done after acid hydrolysis and subsequent neutralization, and therefore, we first investigated the influence in quantity of the neutralization reagents²⁾ on glucosamine. Usually, amino-polysaccharides are hydrolyzed with 6M hydrochloric acid⁴⁾ for several hours at 100°C. After the addition of D-mannitol as the internal standard to a solution of D-glucosamine hydrochloride in 6M hydrochloric acid, the solution was neutralized by three methods. The recovery of glucosamine as glucosaminitol acetate was 25~30% for the neutralization method using silver carbonate or barium hydroxide, and 95~100% for the azeotropic evaporation method. Barium carbonate was not suitable for practical use because it caused the solution to become solidified. Rough neutralization with barium hydroxide often caused alkaline epimerization of glucosamine into mannosamine, and the same observation was true for neutralization with anion-exchange resin, with an accompanying low recovery of glucosamine. Accordingly, it is necessary for a quantitative analysis of amino sugars by GLC to take care with neutralization of the hydrolyzates.

When subjected to a GLC analysis (see Fig. 1) after using the neutralization method of azeotropic evaporation, the yields of amino-alditol acetate obtained by the

hydrolysis of chitin, regenerated chitin and soluble chitin (70% partially acetylated chitin) with 6M hydrochloric acid at 100°C reached their maximum (40~55%) after 20 hr, except for chitosan, in which hydrolysis reached a maximum (42%) after 40 hr. The time course for the hydrolysis of glucosamine hydrochloride with 6M hydrochloric acid at 100°C is shown in Fig. 1. Acid degradation of glucosamine reached 40% after 10 hr, but continued slowly. A similar tendency was observed for the degradation of mannosamine hydrochloride. From these observations, chitin and chitosan are considered to be resistant to hydrolysis because of their fibrous structure. In contrast, the structures of regenerated chitin and soluble chitin are not fibrous but amorphous, and it seems that they were hydrolyzed somewhat more rapidly than chitin and chitosan. On the other hand, the hydrolysis of chitin could hardly be achieved with sulfuric acid (see Fig. 1). The extent of hydrolysis of the chitin derivatives did not exceed 60%, suggesting the existence of a certain competitive relationship between the production of glucosamine and the degradation of glucosamine and aminopolysaccharides.

During the hydrolysis of amino sugars, a by-product was found to appear having a very similar retention time to that of xylitol acetate, which has a slightly shorter retention time by GLC. The molar ratio of this by-product did not exceed 30%, and the same peak was observed in the epimerization mixture of glucosamine with alkali. The appearance of this peak was often, but not always, regardless of the amount of the residual amino sugar. Consequently, it is necessary to pay particular attention to this misleading substance for gas chromatographic analyses of polysaccharides containing amino sugars.

FIG. 1. Hydrolysis of Amino-monosaccharides and Aminopolysaccharides.

In 6M hydrochloric acid at 100°C: □, glucosamine hydrochloride; ■, mannosamine hydrochloride; ○, regenerated chitin; ●, chitin; △, soluble chitin; ▲, chitosan. In 3M sulfuric acid at 100°C: ▼, chitin.

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EXPERIMENTAL

The chitin derivatives used in this study were prepared from the chitin obtained after purifying by the method of Hackman⁵⁾ a practical grade of chitin that was purchased from Sigma Chemical Co. Acid-soluble chitin (70% acetylated chitin) was prepared by the method of Sannan *et al.*⁶⁾ as described by Pangburn *et al.*⁷⁾ Regenerated chitin was prepared as described previously,⁸⁾ and chitosan by the method of Horton and Lineback.⁹⁾

Gas chromatography of the alditol acetates was performed with a Shimadzu GC-5A gas chromatograph equipped with a glass column (3 × 250 mm) of 3% ECNSS-M in Chromosorb W (80 ~ 100 mesh) under isothermal conditions at 193°C, and with a Shimadzu C-E1B chromatopac.

Quantitative analysis of amino sugars. D-Mannitol (0.3 mg, 0.059 mol. eqs.) was added as the internal standard to a 0.1% solution of D-glucosamine or D-mannosamine hydrochloride (6.0 mg, 2.78×10^{-5} mol) in 6 M hydrochloric acid (6 ml). The solution was neutralized with silver carbonate, ion-exchange resin (OH⁻ or HCO₃⁻ form), barium carbonate, barium hydroxide, or by repeated azeotropic evaporation with water. The resulting solution was converted into alditol acetates in the usual manner.^{1,2)}

Degradation of amino-monosaccharides and hydrolysis of amino-polysaccharides with acid. In order to avoid acid-reversion, 0.1% solutions of sugar samples (6 mg) in acid (6 ml) were prepared in test tubes. For the polysaccharides, sugar samples were milled and passed through a 80 Å mesh

before use. The oxygen in the test tubes was replaced with nitrogen to minimize any acid degradation, before each test tube was sealed to give an equal dead space. Degradation and hydrolysis were conducted for the monosaccharides in 6 M hydrochloric acid, and for the polysaccharides in 6 M hydrochloric acid or 3 M sulfuric acid with rotary shaking for several hours at 100°C. Neutralization was effected by azeotropic evaporation for the chloride ion, and by barium hydroxide for the sulfate ion.

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