PROPERTIES OF THE O-GLYCOSIDIC BOND IN O-GLYCOSIDES OF L-SERINE

M. G. Vafina and N. V. Molodtsov

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One of the types of bonds between polysaccharide and protein fragments in glycoproteins is a O-glycosidic bond of β-hydroxy amino acids – serine and threonine with amino sugars: N-acetyl-D-glucosamine and N-acetyl-D-galactosamine.

We have previously investigated the properties of a O-glycosidic carbohydrate–peptide bond for the case of O-glycosides of serine in which the role of carbohydrate component was fulfilled by neutral monosaccharides [1, 2]. It would be interesting to compare the properties of glucosaminides of hydroxy amino acids with the properties of the O-glycosides of serine and to determine how a 2-acetamido group affects the stability of the O-glycosidic bond. In view of this, we have synthesized the O-β-D-glucosaminide of N-benzyloxycarbonyl-L-serine (cbz-L-serine) and have investigated its stability to hydrolytic decomposition.

The condensation of 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-α-D-glucopyranosyl chloride with the methyl ester (ME) of N-cbz-L-serine in dry benzene in the presence of Ag_2CO_3 with the continuous elimination of the solvent by distillation gave a 50% yield of the 2-acetamido-3,4-tri-O-acetyl-2-deoxy-β-D-glucopyranoside of the ME of N-cbz-L-serine. When this compound was treated with a 4 N solution of methylamine in absolute methanol, the 2-acetamido-2-deoxy-β-D-glucopyranoside of the methylamide of N-cbz-L-serine (I) was isolated.

The stability of the O-glycosides of the methylamide of N-cbz-L-serine in 0.4 N HCl at 100°C was found from the increase in the consumption of periodate determined spectrophotometrically at 221 nm [3]. The method of determining the stability of the O-glycosidic bond in an alkaline medium was based on the spectrophotometric determination of aminoacrylic acid derivatives – products of the decomposition of O-glycosides of serine – at 225 nm.

On hydrolysis with 0.4 N HCl, compound (I) decomposed to the extent of 40-43% in 2 h, while the O-β-D-glucopyranoside of the methylamide of N-cbz-serine (II) decomposed to the extent of 47-50%. Compound (I) was hydrolyzed almost completely in 4-6 h, which agrees well with information on the hydrolysis of (II) [2]. On alkaline hydrolysis, the bulk of the (I) and (II) was saponified after 30 min, the rates of decomposition of the two glycosides in 0.1 N NaOH being very similar.

Thus, the conclusion drawn previously for neutral monosaccharides on the insignificant influence of the nature of the monosaccharide on the hydrolytic stability of a O-glycosidic bond [2] also extends to derivatives of D-(amino sugar)s.

Acid Hydrolysis. A standard solution of (I) or (II) or of a solution containing equimolar amounts of the methylamide of N-cbz-serine and N-acetyl-D-glucosamine [or D-glucose for (II)] (0.1 ml) was kept in 0.4 N HCl at 100°C for a predetermined time. The hydrolyzate was treated with 0.4 ml of 0.2 M citrate–phosphate buffer, pH 4.23 and 0.3 ml of 1 mM sodium periodate. The mixture was kept at 100°C for 10 min and was diluted with 5 ml of water, and the absorption was measured at 221 nm. As a blank we used 0.1 ml of 0.4 N HCl, 0.4 ml of buffer, and 5.3 ml of water. The absorption of a sample containing 0.1 ml of the standard solution of (I), 0.4 ml of buffer, and 0.3 ml of a solution of periodate that had been heated at 100°C for 10 min and diluted with 5 ml of water was taken as 0% decomposition.

Alkaline Hydrolysis. To 0.1 ml (1 μmole) of a solution of (I) was added 0.1 ml of 0.2 M NaOH and the mixture was kept the required time and diluted with 5 ml of 50% ethanol, and was then investigated

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spectrophotometrically at 225 nm. A calibration curve was plotted from the absorption of samples containing the methylamide of aminoacrylic acid and N-acetyl-D-glucosamine or D-glucose, according to whether the degree of decomposition of (I) or (II) was being determined.

LITERATURE CITED

1. V. A. Derevitskaya and M. G. Vafina, Izv. Akad. Nauk SSSR, Ser. Khim., 1528 (1967).
2. N. K. Kochetkov, M. G. Vafina, and V. A. Derevitskaya, Izv. Akad. Nauk SSSR, Ser. Khim., 1535 (1967).
3. L. H. Frommhagen, Anal. Chem., 28, 1202 (1956).