HYDROLYTIC STABILITY OF THE ESTER BOND IN AMINOACYL DERIVATIVES OF N-ACETYLGLUCOSAMINE, GLUCOSAMINE, AND N-ACETYLNEURAMINIC ACID

V. A. Derevitskaya and V. M. Kalinevich

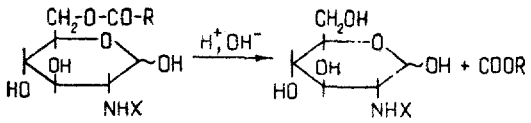
Khimiya Prirodnykh Soedinenii, Vol. 4, No. 1, pp. 28–32, 1968

We have previously synthesized the methyl ester of 9-O-glycyl-N-acetylneuraminic acid [1] and the 6-O-aminoacyl derivatives of N-acetylglucosamine [2] by the carbodiimide method [3]. In the present paper we give data on the stability of these compounds to hydrolysis under a wide range of pH values (pH 1–8).

In order to study the influence of a free amino group in an amino sugar on the hydrolytic stability of the ester bond of aminoacyl derivatives of amino sugars, we also investigated aminoacyl derivatives of glucosamine (base). Glycine, alanine, and ε-aminocaproic acid were used as amino acid components, and diglycylglycine as the peptide. In addition to this, we made a fundamental study of how the properties of the O-aminoacyl derivatives change on passing from 2-deoxy-2-acetamidoglucose and 2-deoxy-2-aminoglucose to 3, 5-dideoxy-5-N-acetylaminononulosic (neuraminic) acid, which is simultaneously an amino sugar, a deoxy sugar, and a keto acid and contains three hydroxy groups in the side chain. As the measure of the stability of the ester bond, we took the percentage decomposition of the aminoacyl derivatives in a definite time at 37° C.

The degrees of decomposition of the 6-O-aminoacyl derivatives of N-acetylglucosamine and of 6-O-glycylglucosamine were measured by the hydroxamic method [4]. The percentage decomposition of methyl 9-O-glycyl-N-acetylneuraminate was determined by the ninhydrin method [4] from the amount of glycine formed in the hydrolysis of the ester bond.

The decomposition of the O-aminoacyl derivatives of N-acetylglucosamine, glucosamine, and N-acetylneuraminic acid under the conditions that we studied took place by the hydrolysis of the ester bond, leading to the formation of an amino acid and an amino sugar.



where $R = -CH_2-NH_2 \cdot {}^1/_2(COOH)_2$, $-(CH_2)_5-NH_2 \cdot {}^1/_2(COOH)_2$, $-CH(CH_3)-CH_2 \cdot {}^1/_2(COOH)_2$, $-CH_2-CO-NH-CH_2-NH_2 \cdot {}^1/_2(COOH)_2$,

here X = H or Ac.

Data on the hydrolysis of the 6-O-aminoacyl derivatives of N-acetylglucosamine and glucosamine (at 37° C) and also literature data on the stability of 6-O-glycylglucose (for comparison) are given in Table 1. As can be seen from the table, in the range pH 4–8 the free amino group exerts a powerful, labilizing influence on the ester bond [compare the stability of 6-O-(N-cbz-glycyl)-N-acetylglucosamine and that of 6-O-glycyl-N-acetylglucosamine].

When the amino group is remote from the ester bond, the stability of the latter rises considerably: 6-O-ε-aminocaproyl-N-acetylglucosamine > 6-O-diglycyl-N-acetylglucosamine > 6-O-glycyl-N-acetylglucosamine. The labilizing influence of an α-amide grouping on the ester bond, which can be seen from the fact that the 6-O-ε-aminocaproyl derivative is more stable than the 6-O-(N-cbz-glycyl)- and 6-O-diglycyl-N-acetylglucosamines, must also be noted.

In the hydrolysis of the aminoacyl derivatives of N-acetylglucosamine in the range pH 1–3, the amino group exerts the opposite effect, i.e., it stabilizes the ester bond: 6-O-glycyl-N-acetylglucosamine > 6-O-diglycyl-N-acetylglucosamine > 6-O-ε-aminocaproyl-N-acetylglucosamine.

An α-amide group likewise apparently has a stabilizing influence on the ester bond since the 6-O-ε-aminocaproyl derivative is less stable than the 6-O-diglycyl and 6-O-(N-cbz-glycyl) derivatives of N-acetylglucosamine.

Thus, the main features found previously for the 6-O-aminoacyl derivatives of glucose [3, 4] are also valid for the 6-O-aminoacyl derivatives of N-acetylglucosamine, and the stabilities of the ester bonds in 6-O-glycylglucose and 6-O-glycyl-N-acetylglucosamine are very similar.

On passing to an amino sugar with a free amino group, the stability of the aminoacyl derivatives changes considerably. While in the pH 1–3 region the rate of hydrolysis of 6-O-glycylglucosamine is comparable with that of 6-O-glycyl-N-acetylglucosamine, in the pH 4–8 region the rate of hydrolysis of 6-O-glycylglucosamine considerably exceeds that of

the 6-O-glycyl derivative of N-acetylglucosamine, which must evidently be due to the influence of a free amino group.

Data on the stability of the aminoacyl derivatives of N-acetyl-glucosamine and the aminoacyl derivatives of N-acetylneuraminic acid (at 37° C) are given in Table 2.

Table 1

Hydrolytic Decomposition of 6-O-Aminoacyl Derivatives of N-Acetylglucosamine, %

Substance	After 24 hr						After 3 hr	After 1 hr
	pH							
	1	2	3	4	5	6	7	8
6-O-Glycylglucose [3]	8	3.5	10	16.5	~50	—	—	—
6-O-Glycyl-N-acetylglucosamine	10	5.5	10.5	16	28	60	40	45
6-O-L-Alanyl-N-acetylglucosamine	13.5	7.2	9.9	13.8	34	62.2	44.5	56.9
6-O-ε-Aminocaproyl-N-acetylglucosamine	20	5	<5	<5	5.9	—	11.2	28.7
6-O-Diglycyl-N-acetylglucosamine	16.7	9.1	12.5	10.2	12	26.4	63.2	67.2
6-O-(N-cbz-Glycyl)-N-acetylglucosamine	17.8	13.3	9	9.5	9.5	10.6	11	36 (3 hr)

It can be seen from the table that all the compounds studied have their optimum stability at pH ~2. The ester bond of an O-aminoacyl derivative of N-acetylneuraminic acid is considerably more labile than the same bond in the aminoacyl derivatives of N-acetylglucosamine and glucosamine, this being shown particularly clearly at pH 1 and 6. This fact, very important from our point of view, must be taken into account in working with mixed biopolymers containing N-acetylneuraminic acid. As is well known, to isolate this acid from a polymer mild acid hydrolysis is used (0.03 N H_2SO_4, 1 hr, 80° C), with the idea that under these conditions no bonds in the polymer other than the ketoside bond of the N-acetylneuraminic acid with the neighboring monosaccharide will be affected. It follows from the results that we have obtained that if the N-acetylneuraminic acid is also linked by an ester bond with other components of the polymer, these bonds must also undergo partial cleavage on mild acid hydrolysis and therefore cannot be detected.

Table 2

Hydrolytic Decomposition of O-Aminoacyl Derivatives of Various Monosaccharides, %

Monosaccharide	After 24 hr						After 3 hr	After 1 hr
	pH							
	1	2	3	4	5	6	7	8
6-O-Glycyl-N-acetylglucosamine (by the hydroxamic reaction)	10.0	5.5	10.5	16	28	60	40	45
6-O-Glycyl-N-acetylglucosamine (by the ninhydrin reaction)	10.7	6.5	10.7	21.5	36.8	55	46.5	45.2
6-O-Glycylglucosamine (by the hydroxamic reaction)	16.3	7	11.5	29	50	75.7	60	57.6 (3 hr)
Methyl 9-O-glycyl-N-acetylneuraminate (by the ninhydrin reaction)	30 (3 hr)	17	32.4	27.2	42.8	48 (6 hr)	46.3 (2 hr)	45.2

Experimental

The hydrolysis of the O-aminoacyl derivatives of the monosaccharides was carried out at 37° C in citrate-phosphate buffer (0.2 M Na_2HPO_4 + 0.1 M citric acid) with pH 8–8.2 and in acetate-hydrochloric acid buffer with pH 1.

Determination of the hydrolytic stability of 6-O-aminoacyl derivatives of N-acetylglucosamine and 6-O-glycylglucosamine (hydroxamic method)

One ml of a 0.01 M solution of the O-aminoacyl derivative of N-acetylglucosamine concerned in a buffer with a given pH was thermostated at 37° C. After equal intervals of time, .02-ml samples were taken and these were made up to 1 ml with a freshly prepared 2 M solution of hydroxylamine having pH 8.5. The mixture was left for 10 min and then, with stirring, 1 ml of 2 N hydrochloric acid and 2 ml of 15% ferric chloride solution in 0.25 N hydrochloric acid were added. To eliminate the gaseous products, the mixture was subjected to vacuum (10 mm) for 30 sec and was then examined in a photocolorimeter at 540 mμ. The percentage decomposition of the starting material after a given time was determined by means of a calibration curve with an accuracy of ±8–10%.

Determination of the hydrolytic stability of methyl 9-O-glycyl-N-acetylneuraminate and 6-O-glycyl-N-acetylglucosamine (ninhydrin method)

A solution of 2 mg of methyl 9-O-glycyl-N-acetylneuraminate in 0.1 ml of an appropriate buffer was thermostated at 37° C. After equal intervals of time, 10–15-γ samples were taken and were deposited on an electrophoregram with a standard solution of glycine in an amount corresponding to 10–15 γ. Electrophoresis was carried out in an acetate buffer at pH 4.5 with a voltage of 900 V for 20 min.

The electrophoregrams were dried in air for 1 hr, treated with a 2% solution of ninhydrin containing $CdCl_2$, dried again in the air for 10 min, and heated in a thermostated oven at 100° C for 15 min. The parts containing the spots corresponding to glycine were cut out from the stained electrophoregram, care being taken that the excised areas of paper were the same. Each section of the paper was cut into narrow strips in such a way that they were not completely separated from one another and were placed in test tubes. Each tube was treated with 5 ml of 40% aqueous methanol and the colored spots were eluted for 2 hr in the dark with vigorous shaking of the tubes after 10–15 min (complete disappearance of the color from the paper at the end of elution). After the end of the elution, the optical density of the colored solutions was measured in a photocolorimeter at 500 mμ. The amount of amino acid in the sample was determined from a calibration curve. The error of the method was ±5–7%.

Agreement of the results obtained by these two methods was checked and found satisfactory in the case of 6-O-glycyl-N-acetylglucosamine.

Summary

The hydrolytic stability of the ester bond of O-aminoacyl derivatives of N-acetylglucosamine, glucosamine (base), and methyl N-acetylneuraminate has been studied at pH 1–8. It has been shown that the stability of the O-aminoacyl derivatives of N-acetylglucosamine is comparable with that of the corresponding glucose derivatives. The presence of a free amino group in glucosamine leads to a considerable labilization of the ester bond. The ester bond of the O-aminoacyl derivatives of methyl N-acetylneuraminate is characterized by a very high lability, and this must be taken into account in working with biopolymers.

REFERENCES

1. V. A. Derevitskaya, V. M. Kalinevich, and N. K. Kochetkov, DAN SSSR, **160**, 596, 1965.
2. N. K. Kochetkov, V. A. Derevitskaya, and V. M. Kalinevich, Izv. AN SSSR, ser. khim., **3**, 496, 1965.
3. N. K. Kochetkov, V. A. Derevitskaya, and L. M. Likhosherstov, Izv. AN SSSR, ser. khim., **4**, 688, 1963.
4. N. K. Kochetkov, V. A. Derevitskaya, and L. M. Likhosherstov, Izv. AN SSSR, ser. khim., **2**, 367, 1967.
5. Zh. V. Uspenskaya and V. L. Kretovich, Methods for the Quantitative Paper Chromatography of Sugars, Amino Acids, and Plants [in Russian], 59, Moscow, 1962.

14 September 1966

Institute of the Chemistry of Natural Compounds, AS USSR