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Abstract

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Study of the Kinetics of Hydrolysis of Some N-Glycosides

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N-glycosides (glycosylamines) are of interest for a number of reasons. Thus, to date we do not have reliable data concerning the tautomerism of their cyclic and acyclic forms, of the Schiff-base type ([1]). Further, the ease of formation of a number of these compounds, for example alkyl- and phenyl-N-glycosides ([2]), suggests the possibility of their formation under biological conditions through direct interaction of sugars with amino compounds. An interesting problem is the preparation of N-glycosides of medicinal substances, since glycosylation increases solubility ([3]), decreases toxicity, and in some cases changes the nature of the action ([4]). The ease of hydrolysis, however, is a serious obstacle to the use of N-glycosides. From this point of view, it is of interest to study the kinetics of their hydrolysis as a function of structure. Such a study, moreover, with the accumulation of data, will provide material for understanding the mechanism of hydrolysis of N-glycosides, and also for elucidating the conformations of the sugar residue.

Table 1

Determination of the hydrolysis constant of *p*-carboxyphenylglucosylamine (initial concentration of N-glycoside 6 mg/ml)

Duration, min	Cleavage of N-glycoside, %	$K \cdot 10^3$
90	17.29	2.11
151	29.98	2.36
210.5	37.01	2.20
270.5	46.70	2.33
330.5	49.58	2.07
390	55.92	2.11
452	62.26	2.15
510	65.72	2.10

K (avg.) $= (2.18 \pm 0.026) \cdot 10^{-3}$ min^{-1}
$t_h = 5$ h 18 min.

Table 2

Determination of the hydrolysis constant of *p*-carboxyphenylgalactosylamine (initial concentration of N-glycoside 6 mg/ml)

Duration, min	Cleavage of N-galactoside, %	$K \cdot 10^{-2}$
36	29.40	0.97
59.5	47.85	1.09
90.5	63.42	1.11
120.5	74.37	1.13
150	79.56	1.06
180.5	81.86	0.95

K (avg.) $= (1.05 \pm 0.021) \cdot 10^{-2}$ min^{-1}
$t_h = 66$ min.

In the present work we set ourselves the goal of studying the relative stability of the N-glycosidic bond of glycosylamines as a function of the sugar component constituting them. In choosing an aglycone for obtaining glycosylamines, we selected *p*-aminobenzoic acid, which is important for the preparation of a number of pharmaceutical preparations.

There are relatively few works in the literature on the study of hydrolysis of N-glycosides. For these investigations, in most cases, the polarimetric method was used ([5–10]). This method, however, can hardly give sufficiently reliable results, since the change in the values of the specific rotation of solutions may in this case be associated not only

with the hydrolysis of N-glycosides, but also, possibly, with the interconversion of cyclic and acyclic forms of the glycosides themselves. Therefore we developed a method for studying the hydrolysis of glycosylamines by colorimetric determination of the aglycone—amine—formed during hydrolysis. The essence of the method consists in rapid extraction of the aglycone with ether, its coupling with *p*-nitrophenyldiazonium, and colorimetric determination of the dye formed from an empirical curve (see the experimental part). The method described by us is more laborious than the polarimetric one, but it eliminates possible errors associated with tautomerism of N-glycosides and, moreover, can be used in cases of small optical rotations of glycosides at low concentrations (in cases of low solubility).

Table 3

Determination of the hydrolysis constant of *p*-carboxyphenyl-xylosylamine (initial concentration of N-glycoside 3.39 mg/ml)

Duration, min	Cleavage of N-xyloside, %	$K \cdot 10^{-2}$
21.5	21.96	1.15
39.5	38.04	1.21
60	56.19	1.37
80	65.18	1.34
100	70.89	1.23
120	76.59	1.21
150	82.13	1.15

$$K \text{ (avg.)} = (1.23 \pm 0.021) \cdot 10^{-2} \text{ min.}^{-1}$$

$$t_h = 56 \text{ min.}$$

Table 4

Determination of the hydrolysis constant of *p*-carboxyphenyl-arabinosylamine (initial concentration of N-glycoside 2.55 mg/ml)

Duration, min	Cleavage of N-arabinoside, %	$K \cdot 10^{-2}$
14	23.06	1.87
21	32.93	1.91
29	39.2	1.72
39.5	49.58	1.73
49	59.96	1.87
59.5	64.57	1.75
69.5	70.33	1.75

$$K \text{ (avg.)} = (1.80 \pm 0.020) \cdot 10^{-2} \text{ min.}^{-1}$$

$$t_h = 38.5 \text{ min.}$$

We applied the developed method for determining the aglycone, *p*-aminobenzoic acid, to the study of the kinetics of hydrolysis of *p*-carboxyphenyl-glycosylamines with different sugar components: D-glucose, D-galactose, D-xylose, and L-arabinose. Taking into account the instability of N-glycosides, we studied the hydrolysis kinetics without the addition of acids or alkalis, at 25°. The hydrolysis constants were calculated by the Arrhenius equation for first-order reactions. The mean probable errors of the result and the half-life periods (t_h)

reaction scheme: chair-form N-glycoside hydrolysis through protonated intermediate and oxonium ion to sugar hydroxyl product

Figure 1: reaction scheme: chair-form N-glycoside hydrolysis through protonated intermediate and oxonium ion to sugar hydroxyl product

were also calculated. The results of the experiments are presented in Tables 1-4.

Comparing the average values of the hydrolysis constants of *p*-carboxyphenyl-glycosylamines formed by different sugars, one may conclude that the glycosylamines studied can be arranged in the following order according to their stability: glucosylamine > galactosylamine > xylosylamine > arabinosylamine.

It is interesting that the same sequence with respect to resistance to hydrolysis is also observed in the case of O-glycosides—phenyl glycosides and *p*-chlorophenyl glycosides ([11]). For O-glycosides such a sequence is determined by the conformational features of the glycosides, galactosides, and xylosides ([12]). Most probably, in the case of the hydrolysis of N-glycosides we are dealing with the same chair conformations, and their hydrolysis proceeds according to the same schemes:

In favor of this are also the considerations that it is difficult to imagine that such an easily cyclizing residue as the glucose residue could exist-

exist not in the chair conformation, but in an open form of the Schiff-base type. On the other hand, the comparatively similar values of the hydrolysis constants obtained for glucosides, galactosides, xylosides, and arabinosides speak in favor of their conformational similarity. These observations, of course, are only tentative and must receive further confirmation.

Experimental Part

The syntheses of the N-glycosides were carried out by Sorokin' s method (13, 14), somewhat improved by other investigators (15, 16), in yields of 60–70%. The constants of the compounds obtained were close to those known in the literature; for example, m.p. of *p*-carboxyphenyl-*D*-glucosylamine, 132°; m.p. of *p*-carboxyphenyl-*D*-galactosylamine, 152–153°; m.p. of *p*-carboxyphenyl-*D*-xylosylamine, 172°; m.p. of *p*-carboxyphenyl-arabinosylamine, 154–155°.

Development of a colorimetric method for studying hydrolysis kinetics by cleavage of the aglycone–amino compound

The difficulty in studying the hydrolysis process of glycosylamines, in contrast to that of O-glycosides, lies in the fact that it is not possible to interrupt the hydrolysis process, since glycosylamines are hydrolyzed by water. Therefore, in order to determine the amount of aglycone formed at a given stage of the reaction, it must be separated from the hydrolysate as rapidly as possible. Having tested a

number of solvents, such as ether, benzene, and carbon tetrachloride, we settled on extraction of the aglycone with ether. A series of experiments was carried out to establish the optimal conditions for extracting *p*-aminobenzoic acid from aqueous solutions of various concentrations. The following were varied: the amount of ether, the number of extractions, the extraction time, and the use of salting-out. Taking into account the need for the most rapid extraction possible, we found it possible to limit ourselves to a single extraction by increasing the amount of ether, with a short extraction time. To achieve greater completeness of extraction, the solution must first be saturated with sodium chloride. With this method, for solutions of various concentrations of *p*-aminobenzoic acid, containing from 0.27 to 2.7 mg/ml of solution, the unextracted residue amounts to from 3 to 6%. Parallel experiments give satisfactorily concordant data.

Specially designed experiments showed that the method for determining the amine by diazotization is insensitive at low concentrations; determination of the amine by weight in the form of a sparingly soluble dye requires a long time. Therefore we settled on determining the aglycone by a colorimetric method, by converting it into a soluble dye and determining the amount of dye formed from the optical density of the resulting solutions. For coupling as the diazo component we chose *p*-nitrophenyldiazonium, since it is sufficiently stable in the form of the diazotate.

In developing the conditions for coupling *p*-aminobenzoic acid with *p*-nitrophenyldiazonium, the amounts of reagents were varied: *p*-nitrophenyldiazonium, sodium acetate, and caustic soda, as well as the temperature and duration of the reaction at each stage. These conditions (see below) were used in the subsequent experiments.

For the determination of *p*-aminobenzoic acid, a conditional experimental curve was obtained, constructed according to data obtained under the same conditions as in the hydrolysis of the glycosides. For this purpose, a series of solutions of *p*-aminobenzoic acid of different but precisely known concentration (from 0.3 to 24 mg/ml) was subjected to extraction with ether, coupling, and colorimetry. Use of the curve obtained eliminates the introduction of any corrections (for example, for incomplete extraction).

Procedure for conducting experiments on the study of hydrolysis kinetics

Accurately weighed portions of glycosides (depending on solubility, see Tables 1–4) were dissolved in 100 ml of water in volumetric flasks with constant shaking at an ultrathermostat temperature of $25 \pm 0.05^{\circ}$. To determine the constants, 6–8 samples of 10 ml each were taken at various time intervals. Each sample was placed in a separatory funnel, to which 2.3 g of finely ground sodium chloride and 40 ml of ether were added. The mixture was shaken vigorously for 4 min; then the aqueous layer was drained off, and the ether layer was quantitatively transferred to a 100-ml volumetric flask. The moment of sampling was taken to

be the moment of separation of the layers after extraction of the solutions. The ether was then distilled off, and the residue of *n*-aminobenzoic acid was dissolved in hot water (about 60°). After the solution had reached room temperature, the liquid was brought to the mark.

To convert it into a dye, 10 ml of each solution of the extracted *n*-aminobenzoic acid was placed in a 250-ml flask and, after cooling to 5°, mixed with 10 ml of a solution of *n*-nitrophenyldiazonium and 10 ml of a 10% solution of sodium acetate, after which the mixture was kept at 5° for 20 min. Then 1.3 ml of a 10% NaOH solution was added to the flask, and the mixture was kept at 5° for 15 min. After this the solution was brought to the mark and colorimetered in an FEK-M photoelectric colorimeter with a blue light filter, 470 mμ, and a layer thickness of 0.5 cm.

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Note: Figure translations are in progress. See original paper for figures.