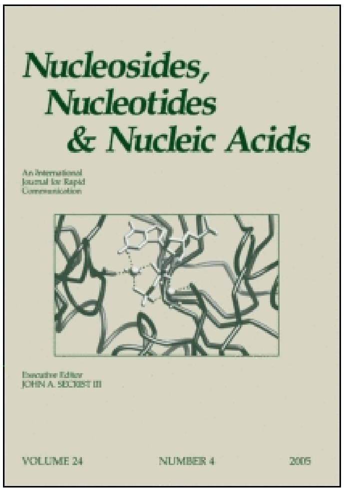

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The Acidic Hydrolysis of Nucleosides and Nucleotides

Ewa Zielonacka-lis[a]
[a] Institute of Bioorganic Chemistry Polish Academy of Sciences Poznań, Noskowskiego, Poland

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THE ACIDIC HYDROLYSIS OF NUCLEOSIDES AND NUCLEOTIDES

Ewa Zielonacka-Lis

Institute of Bioorganic Chemistry
Polish Academy of Sciences
Poznań, Noskowskiego 12/14, Poland

ABSTRACT: The influence of sugar and base substitutions on the stability of the N-glycosidic bond of nucleosides in acidic medium is examined in detail. Then the mechanistic aspect of the phenomenon is described. The last part is devoted to the acidic hydrolysis of nucleotides.

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1. Introduction.

The problem of stability of the nucleoside and nucleotide glycosyl bonds is one of the crucial issues of biology and chemistry.

Regulation of the breaking and conservation processes of the glycosyl bonds is relevant to the proper functioning of all living organisms. By way of glycosyl bond breaking, spontaneous or induced depurination and depyrimidination of DNA take place. The resultant apurinic and apyrimidinic sites become a potential source of mutagenic changes. Moreover, incorrect bases are introduced to DNA as a consequence of the action of unfavourable extra- and intracellular factors. One of the most important modes of removing undesired elements from DNA is the action of glycosylases - repair enzymes, which catalyze the cleavage of base - sugar bonds in altered or damaged nucleoside residues[1]. Thus, it is extremely significant that the conditions causing hydrolysis of these bonds, and the chemical principles involved be defined precisely.

An appropriate regulation of glycosyl bond stability is often used in synthetic practice with respect to nucleosides in the deoxy series[2-4]. The differences in the stability of this bond are employed in DNA sequencing[5].

Under the acidic conditions almost all hitherto tested nucleosides break into the purine or the pyrimidine heterocyclic base and the sugar, i.e. ribose or deoxyribose.

2. Structural factors influencing the stability of the nucleoside glycosyl bond in acidic medium.

2.1. The sugar moiety effect.

Increasing number of hydroxyl groups in the sugar moiety leads to greater stability of nucleosides under acidic conditions.

The 2'-hydroxyl group appears to have a particularly strong stabilizing effect on acid hydrolysis rate, and for this reason deoxynucleosides hydrolyze at least 100 times faster than ribonucleosides[6-10]. This fact used to be

TABLE 1. Apparent first-order rate constants (10^5k, sec^{-1}) for hydrolysis in 0.10 M HCl, 80°C

adenosine	16.5
2'-deoxyadenosine	12500
3'-deoxyadenosine	91.6
2',3'-dideoxy-2',3'-didehydroadenosine	35000
adenine arabinoside	22.0
adenine xyloside	11.0
2'-C-methyladenosine	5.00

explained in terms of the negative inductive effect of the hydroxyl group, which by withdrawing electrons hinders the slow step of the reaction[8]. This effect occurs irrespectively of the nature of aglycone[7,11,12] and furanosyl[7,11] or pyranosyl[12] as the glycosyl moiety.

Nucleoside stability is also increased by the presence of the 3'-OH group, although to a much smaller degree than when this substituent is introduced in position 2'[10,13]. The observed first-order rate constant of the acidic hydrolysis of 3'-deoxyadenosine is about 5-7 times greater than for adenosine under analogous conditions (TABLE 1)[6]. Similarly as in the case of O-glycosides, stabilizing effects additivity can also be observed.

The configuration of hydroxyl groups on C-2' and C-3' exerts rather weak influence - the rate of hydrolysis in acidic medium is changed by a few times[10,13]. The stability order established for the adenine nucleosides is the following: xyloside is more stable than riboside, which in turn is more stable than arabinoside (TABLE 1)[6].

Substitution of the 2'-OH group by a more electron withdrawing substituent causes further stabilization of the glycosyl bond[10], and thus for instance 2'-O-p-nitrobenzenesulfonyladenosine is stable under conditions in which adenosine is hydrolyzed[14]. The introduction of acetyl groups into

TABLE 2. Apparent first-order rate constants (10^4k, sec^{-1}) for hydrolysis in 0.1 N HCl, 97.5°C

adenosine	10
5'-O-methyladenosine	10
3'-O-methyladenosine	7.5
2'-O-methyladenosine	6.0
2',3'-di-O-methyladenosine	3.5

sugar moiety in positions 2', 3' and 5' of an unusually labile system of hypermodified nucleoside wyosine (see pp 6-9) causes an about eighty-fold slow-down of the acidic hydrolysis reaction[15].

Introduction of a methyl group instead hydrogen at C-2' of adenosine sugar moiety as exemplified by 2'-C-methyladenosine decreases the solvolytic rate constant to about one--third of the value for adenosine under identical conditions (TABLE 1)[6]. As shown in TABLE 2[16], replacement of the adenosine 2'- and/or 3'-hydroxyl group by a methoxyl group slightly stabilizes the glycosyl bond to acid-catalyzed hydrolysis.

Methylation of uridine O-2' and O-3' results in three- to sevenfold decrease of hydrolysis rate[17].

Ketonucleoside, psicofuranine is hydrolyzed 10^3-10^4 times faster than adenosine. This rate difference is similar to that found with aldo- and ketoglycosides[13].

2.2. The basic part.

2.2.1. Purine nucleosides vs pyrimidine nucleosides.

There is a considerable difference in the stability of the glycosyl bonds in purine and pyrimidine nucleosides[10,13]. For purine nucleosides, the process of glycosyl bond breaking proceeds much faster in acidic than in neutral medium. However, the hydrolysis rates of pyrimidine nucleosides are more pH independent and for instance the rate of deoxycytidine hydrolysis remains unchanged in pH range

TABLE 3

	pH	temp. °C	$t_{1/2}$
3-methylguanosine	2.1	25	7 min[22]
	0.1M HCl	25	42 sec[22]
3-methyladenosine*	1	25	17 min[23]
3-methylinosine	3	40	reaction completed after 24 h[24]
	1	room temp.	reaction completed after 10 min[24]

*in form of a salt of toluene-p-sulphonic acid (TsOH)

1-4[7,18], whereas deoxyadenosine and deoxyguanosine are nearly 1000 times more stable at pH 4 than at pH 1[6-8,19]. This is also the case with thymidine, deoxyuridine and 5-bromodeoxyuridine which at pH 7 show the same hydrolysis rate as at pH 3[20]. Thus, the great difference in glycosyl bond stability between purine and pyrimidine nucleosides in acidic medium is much less marked at higher pH.

2.2.2. Substituents in the basic part of purine nucleosides.

Substituents introduced at position 1 and 2 of purine nucleosides do not exert significant influence on the stability in acidic medium[6,21].

On the other hand, substituents at position 3 of purine nucleosides cause considerable acceleration of acid hydrolysis, as shown in TABLE 3. This pertains to 3-methylguanosine, 3-methyladenosine and 3-methylinosine.

Observations taken during synthesis (substitution of a purine at N-3 by a removable blocking group enabled the preparation of 7-substituted purines[25,26]) indicate a great molecular overcrowding caused by the presence of the substituent in this position.

Also tricyclic Y nucleosides, which are very labile in acidic medium, contain a structural unit of 3-methylguanosine (FIG. 1).

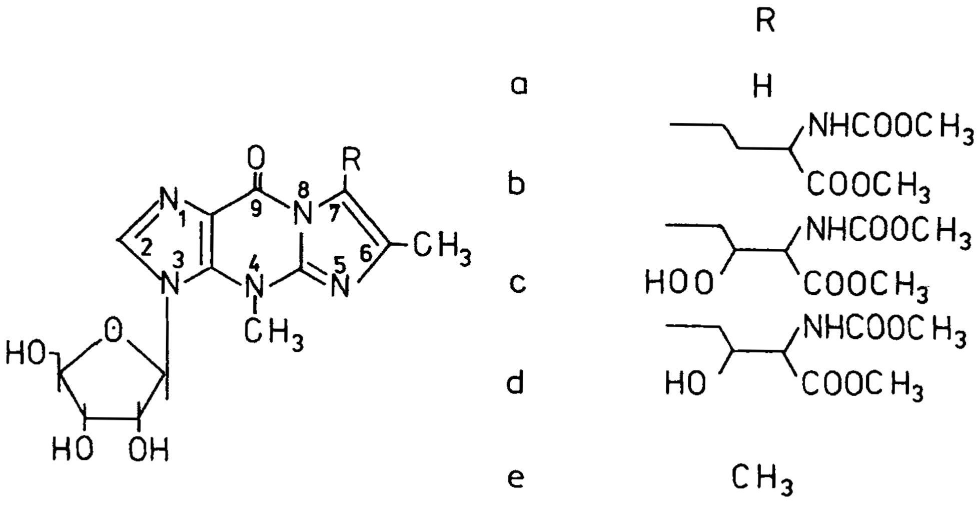


FIGURE 1

TABLE 4

pH	temp. °C	$t_{1/2}$
0.1M HCl	25	95 sec
2.1	25	19 min*
2.9	37	41 min*

*no information about the value of the error

TABLE 5. Apparent first-order rate constants (10^4k, sec^{-1}) and half-lives (h) for hydrolysis of wyosine

	temp. °C	23	30	37
pH				
2.90		0.542 (3.55)	0.857 (2.25)	2.228* (0.86)
3.65				0.343 (5.61)
4.40				0.051 (37.46)

*coefficients of variation are in the range of 6-8%

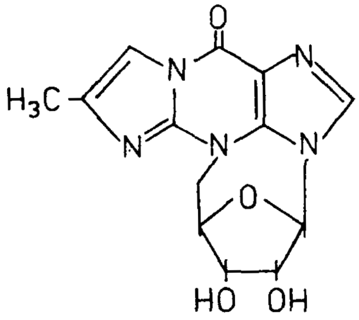


FIGURE 2

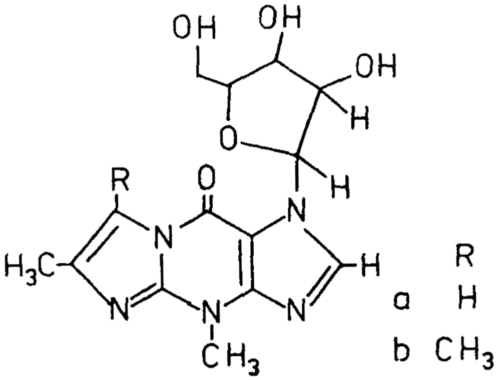


FIGURE 3

Data pertaining to the stability of the glycosyl bond in wyosine (FIG. 1a) are as follows[22].

A pH and temperature dependence of the reaction rate constants of the acidic hydrolysis of wyosine was observed (TABLE 5)[27].

The pH dependence of decomposition rate constants of the glycosyl bond in wyosine is linear. and no general acid-base catalysis was observed similarly as in the case of previously investigated nucleosides[6,8]. Measurements of reaction rate constants at various temperatures served to determine the entropy of activation[27].

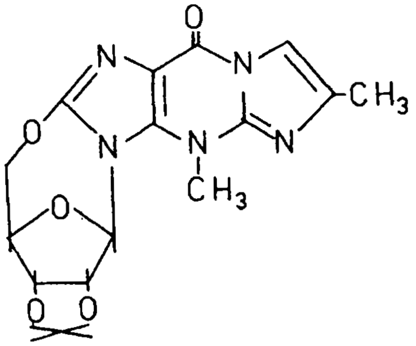


FIGURE 4

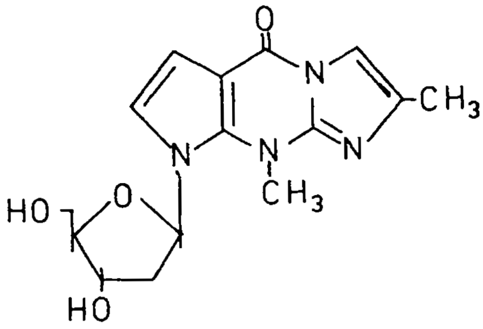


FIGURE 5

So far, such a great lack of stability of the glycosyl bond has not been observed in the ribo series of compounds. To account for this fact in the Y nucleosides, the synthesis of their various, sometimes remote analogues with hard to define changes in the electron and spatial structure was undertaken. The stability of the glycosyl bond was determined for each of the compounds, which turned out to be many times more stable than wyosine. This is shown in TABLE 6.

Glycosyl bond stability was also studied in 4-demethylwyosine and 5-methyl-4-demethylwyosine[15,32]. On the basis of obtained results and the Arrhenius equation it was stated that at 25°C in 0.1 M HCl the hydrolysis rate for these analogues is 10^6 times lower in comparison with wyosine[32].

TABLE 6

	pH	temp. °C	time, h	% of hydrolysis
4,5'-cyclo-3-β-D-ribofuranosyl-4,9-dihydro-6-methyl-9-oxoimidazo [1,2-a]purine (FIG. 2)	0.09M HCl	37	68	0[28]
	0.9M HCl	37	27	~30[28]
1-ribofuranosyl-4,9-dihydro-4,6-dimethyl-9-oxo-imidazo [1,2-a] purine (one of the anomers) (FIG. 3a) and 1-ribofuranosyl-4,9-dihydro-4,6,7-trimethyl-9-oxo-imidazo [1,2-a] purine (one of the anomers) (FIG. 3b)	2.9	37	72	0[29]
	2.9	95		0[29]
	2N HCl	37		0[29]
	0.5N HCl	85-90	10	100[29]
2,5'-cyclo-2-oxo-2',3'-O-isopropylidenewyosine (FIG. 4)	2.9	37	24	0[30]
	2.1	25	24	0[30]
	0.1M HCl	50	20	100[30]
	1M HCl	30	$t_{1/2}$=30 min[30]	
N-1-deaza-2'-deoxy-wyosine (FIG. 5)	0.1N HCl	room	overnight	0[31]
	0.1N HCl	70	24	100[31]

The decomposition rates of the glycosyl bond in wyosine and its ethyl derivative were compared under identical conditions[15]. The same was also done for 3-methyl-, 3-ethyl-, and 3-isopropylguanosine[32]. From the obtained results it follows that a violent breaking of the glycosyl bond in the Y nucleosides in acidic medium is to a significant extent caused by the tendency to reduce the steric interaction between the methyl in position 4 and the ribofuranosyl group in position 3.

The hydrolysis rate of 6-substituted adenosines resembles that of adenosine[6], it is also true for ureido-

TABLE 7

7-methyladenosine	222
7-ethyladenosine	169
N^6-methyladenosine	0.82*
3-methyladenosine·TsOH	4000
1-methyladenosine	0.56*

*on the basis of the Arrhenius equation (55.0-80.0°C)

-purine hypermodified nucleosides: N^6-(N-threonylcarbonyl)-adenosine and N^6-methyl-N^6-(N-threonylcarbonyl)adenosine (only a few-fold changes in the decomposition rate were observed in 1 N HCl, 37° and 30°C)[33].

In the case of the monocations of 6-substituted 9-(β-D-ribofuranosyl)purines the spontaneous decomposition is markedly accelerated upon decrease of substrate basicity, i.e. when the electron-density in the purine ring is reduced. This has been proved for the following set of nucleosides: 6-chloro-9-(β-D-ribofuranosyl)purine and 6-methoxy-9-(β-D-ribofuranosyl)purine compared with adenosine and N^6-dimethyladenosine[34].

The rate constants (10^5k, min^{-1}) obtained for 7-alkyladenosine and its isomers in 0.1 N HCl, 25°C were as below (TABLE 7)[35].

Therefore, the order of stability of the glycosyl bond in substituted adenosines can be presented by the following sequence: 3-methyladenosine $<$ 7-methyladenosine $\ll N^6$-methyladenosine $\lesssim$ 1-methyladenosine.

The introduction of the methyl group to the N-7 of deoxyguanosine caused significant labilization of the glycosyl bond of this compound at high pH[8,10]. The reactivities of 7-methylguanosine and 1,7-dimethylguanosinium iodide at high pH (in the pH range where 7-methylguanosine is a monoprotonated leaving group) are very similar. At low pH, where a dication is formed, guanosine, 7-methylguanosine and 1,7-dimethylguanosinium iodide hydrolyze at essentially identical rates; the reactivity of the natural and methylated guanine ribosides and deoxyribosides is similar[8].

Contrary to 8-bromoguanosine and 8-bromoadenosine and compared to the parent nucleosides, 8-aminoguanosine, 8-monomethylaminoguanosine and 8-methoxyadenosine hydrolyze slower in acidic medium[6,36]. A 110-fold rate acceleration of hydrolysis was observed for 8-N(CH$_3$)$_2$-adenosine as compared with 8-NHCH$_3$-adenosine and 700-fold rate acceleration of 8-N(CH$_3$)$_2$-guanosine compared to 8-NHCH$_3$-guanosine. It was stated that both fast hydrolyzing compounds occur in the syn conformation, while the remaining ones are a mixture of syn and anti[36].

Except guanosine and 7-(β-D-ribofuranosyl)guanine, all hitherto tested purine 7-β-D-ribofuranosides hydrolyze easier than the isomers of 9. The ratio of hydrolysis rate of *N*-7/*N*-9 in 1.02 *N* HCl at 25°C equals 33.8 for adenosine and its 7 isomer, 22.7 for 6-methylamino-9-(β-D-ribofuranosyl)purine and its isomer 7, 19.5 for the pair: 6-dimethylamino-9-(β-D-ribofuranosyl)purine and 6-dimethylamino-7-(β-D-ribofuranosyl)purine, but only 2.5 for nebularine and its 7 isomer, and 1.5 for the pair: inosine, 7-(β-D-ribofuranosyl)hypoxanthine[37].

2.2.3. Acidic hydrolysis of pyrimidine nucleosides.

The alkylation of pyrimidine nucleosides in the O-2 position has a strongly destabilizing influence on the glycosyl bond: O^2-alkyldeoxycytidine is 10^4 times more labile than deoxypurine nucleosides, O^2-alkyldeoxyuridine and thymidine are also more labile than deoxypurine nucleosides. The introduction of alkyl groups in position O-2 does not affect the ratio of rate constants resulting exclusively from the nature of the sugar. The glycosyl bond stability of all O^4-alkylated deoxyribo- and ribopyrimidine nucleosides resembles that of non-modified compounds[38].

Deoxycytidine hydrolizes easier than deoxyuridine derivatives[10]. The introduction of a methyl group into deoxycytidine in position C-5 bears no significant influence on the rate of glycosyl bond breaking[39,40], but the hydrolysis rate of 5-bromodeoxycytidine is approximately 100 times greater than for deoxycytidine and 5-methyldeoxycytidine[18].

In the case of deoxyuridine the acid-catalyzed hydrolysis is accelerated both by electron donor and electron acceptor substituents in position 5[10,11,13,20].

To the small group of pyrimidine ribonucleosides which with an appreciable rate hydrolyze in diluted acetic acid belongs isocytidine[10].

Dihydropyrimidine nucleosides hydrolyze more rapidly than the corresponding unhydrogenated compounds[10,13].

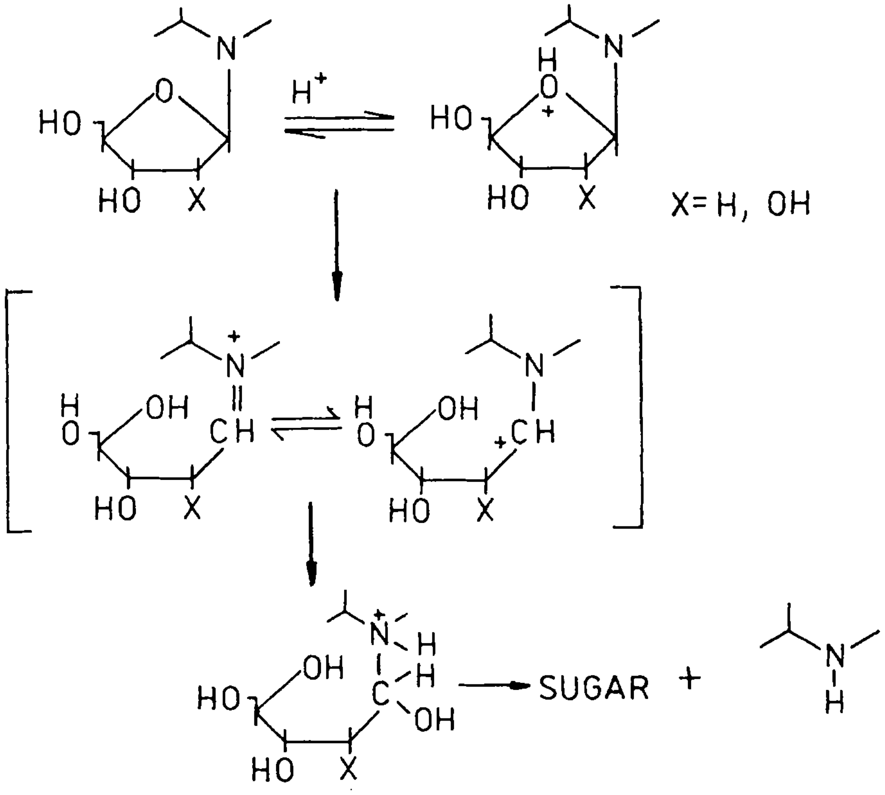
3. The mechanism of the acidic hydrolysis of nucleosides.

In the first years of the 1960's it was accepted that the mechanism of acidic hydrolysis of nucleosides is analogous to simple glycosylamines[41]. In that case the essential steps of reaction could consist in: protonation of the sugar ring oxygen (or when the first proton adds to a nitrogen atom of a heterocyclic base, intramolecular proton transfer to the annular oxygen of the sugar[42]), opening of the sugar ring, attack of the water molecule on the newly formed Schiff's base resulting in a heterocyclic base and a sugar (SCHEME 1). The credibility of this reconstruction was seriously questioned in the next years[13,20]. It was stressed[13] that the important factor which facilitates ring opening with glycosylamines is mesomeric electron release of the amino nitrogen. However, in the case of nucleosides the nature of the nitrogen atom participating in the glycosyl bond formation is different.

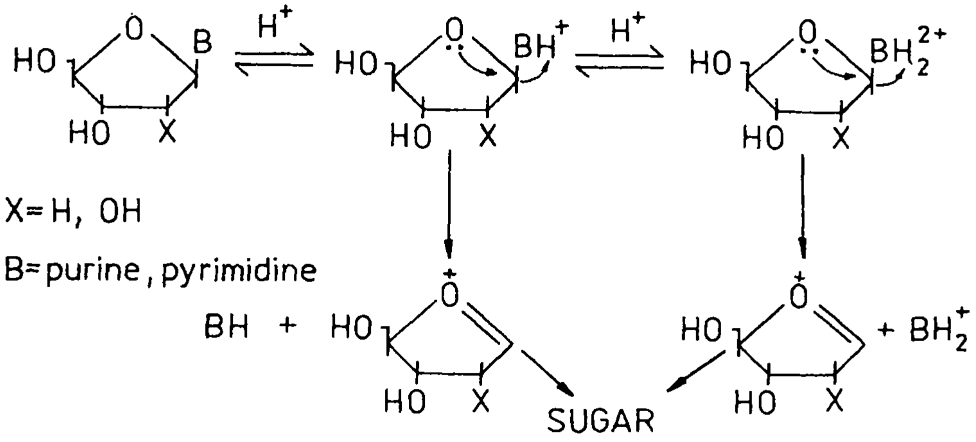
At present, it is claimed that hydrolysis of the glycosyl bond in nucleosides proceeds according to SCHEME 2 - the protonation of the heterocyclic part of the molecule being followed by slow glycosyl bond breaking.

To determine the mechanism of the acid hydrolysis of nucleosides it is necessary to establish:

1. whether the breaking of the glycosyl bond involves the breaking of the C-N or C-O bond, thus, if the hydrolysis



SCHEME 1



SCHEME 2

proceeds without affecting the sugar part, or through Schiff's base,

2. whether the process is uni- or bimolecular.

Data analysis can provide solutions to these problems.

3.1. Data supporting the lack of breaking of the C-O bond.

3.1.1. The pH-rate profiles.

Observation of the pH dependence of reaction rate constants supplies valuable information on the course of the reaction. Substituted benzylidene-t-butylamines containing electron-withdrawing groups[43], as well as N-arylglucosylamines[44,45] form cationic Schiff's bases in the course of hydrolysis, and in both cases the nature of the dependence between the reaction rate and pH is the same: with the growth of acidity, the reaction rate increases up to a certain maximum, and then begins to decrease. No such maximum is observed in the course of nucleoside hydrolysis[6,8,18,34]. It is also true for hypermodified Y nucleoside - wyosine[22,27,32].

3.1.2. The lack of anomerization.

In the course of a reaction which proceeds with the breaking of the C-O bond anomerization could be expected, but in a majority of experiments on the acidic hydrolysis of nucleosides this effect has not been observed[13]. A detailed study of guanosine, 8-NH_2-guanosine, 8-$NHCH_3$-guanosine, 8-$N(CH_3)_2$-guanosine and 8-Br-guanosine[36] did not show anomerization in any of the substrates. Also in the case of an acid hydrolysis of 6-subsituted 9-(β-D-ribofuranosyl)purines no anomerization of substrates competitive with hydrolysis was stated[34]. However, one observation made during the hydrolysis of thymidine and 2'-deoxyuridine in 2 M $HClO_4$ at 90°C is worth mentioning[46]. It was stated that both underwent anomerization ($\beta \rightleftharpoons \alpha$) and isomerization (furanoside $\rightleftharpoons$ pyranoside). It is interesting that under identical conditions these phenomena were stated neither for 2'-deoxycytidine nor 5-bromo-2'-deoxyuridine[46].

3.1.3. The electron effects of substituents at the sugar and heterocyclic base.

The donation of electrons by substituents in the heterocyclic part of the purine nucleosides in position 8[36] and 6[34,37] reduces the rate of glycosyl bond breaking. On the other hand, the withdrawal of electrons accelerates the process[34,36]. This fact can be explained by mechanism 2, because if the reaction proceeded via Schiff's base, contrary results would be expected. Theoretical calculations[47] showed that the presence of amino group at a carbon in position 8 increase the electron density at N-9, whereas the presence of chloride in the same position decreases it in comparison with guanine. The electron density at N-9 is reduced upon protonation at N-7.

3.1.4. Experiments on 7-methylguanosine, 7-methyl-2'-deoxyguanosine and 1,7-dimethylguanosinium iodide.

At low pH the reactivity of the pair: 7-methylguanosine and guanosine is similar like in the case 7-methyl-2'-deoxyguanosine and 2'-deoxyguanosine[8]. Here the rationale is that under the conditions of dication formation the difference of basicity of unsubstituted and methylated substrates vanishes almost completely. What is more, the presence of the proton or the methyl group bear negligible influence on the nature and properties of guanine skeleton as the leaving group.

Alike reactivity of the iodide of 1,7-dimethylguanosine and 7-methylguanosine in weak acidic environment[8] can also be explained on the basis of mechanism 2. In the pH range in which 7-methylguanosine is a monoprotonated leaving group, the characteristics of these two compounds are expected to be nearly the same, and thus the reaction rates are similar too.

3.1.5. The influence of sugar hydroxyl groups.

The stabilizing influence of the hydroxyl groups in the sugar part of the molecule can be accounted for by mechanism 2. The inductive effect of these groups inhibits the slow step of acidic hydrolysis - the breaking of the C-N bond of protonated nucleoside[6,8]. However, even if hydrolysis proceeded via mechanism 1, the hydroxyl groups in the sugar part would still hinder the process[10,13].

The above experimental data can be explained by mechanism 2 which assumes the protonation of the heterocyclic part of the molecule, followed by the breaking of the C-N bond.

3.2. Experimental data differentiating mechanisms A-1 and A-2.

3.2.1. The value of the entropy of activation.

In acid catalyzed reactions of hydrolysis the entropy of activation ($\Delta S^{\neq}$) can serve as a criterion differentiating between mechanisms A-1 and A-2[48]. If the reaction proceeds according to mechanism A-1 the $\Delta S^{\neq}$ value is much greater than in the case of reaction A-2 - all reactions of the A-1 type are characterized by low entropies either with a positive or negative sign, whereas A-2 reactions by great negative values of the entropy of activation[49]. In acid catalyzed reactions of nucleoside hydrolysis the values of the entropy of activation usually approximates zero[6,27,37] indicating that the reaction proceeds via mechanism A-1.

3.2.2. Secondary deuterium isotope effects.

The secondary deuterium isotope effect was measured in the hydrolysis reaction of inosine and adenosine dications and inosine monocation[50]. The determined value of ~1.20 shows that these reactions occur via transition states of considerable carbonium ion character. The simplest interpretation is that the reactions are classical A-1 involving unimolecular decomposition of the protonated substrate to yield free oxocarbonium ion as an intermediate. Since the experimentally determined value of the secondary deuterium isotope effect does not distinguish between C-N and C-O bond cleavage in the transition state, theoretical calculations were undertaken[51]. Together with experimental results, they led to the conclusion that in the transition state the C-N bond is largely or completely cleaved.

3.2.3. Bunnett's constant.

Bunnett's value (constant w[52] in the equation:

$$\log k + H_o = w \log a_{H_2O} + \text{const}$$

where k is the rate constant, H_0 is Hammett's acidity function and a_{H_2O} is the activity of water) was determined[53] for the hydrolysis reactions of guanosine and 7-methylguanosine. The fact that it is close to zero, excludes the participation of a water molecule in the rate-limiting step of the reaction.

3.2.4. The steric effects in 7- and 9-β-D-ribofuranosylpurines.

Changes in the rate of hydrolysis which result from a different site of substitution of the sugar part relatively to purine (N-7 or N-9) can serve as a criterion for distinguishing mechanisms A-1 and A-2 in cases when bulky substituents occur in position 6. In the A-2 type of reactions the presence of bulky groups near the reaction centre causes reaction slow-down, whereas a higher rate of A-1 reactions results from steric acceleration. For some isomers of 7, a 20--30 times increase of the reaction rate is observed in comparison with isomers of 9[37]. Such an increase is probably due not only to electron factors but also an additional steric acceleration effect. It can therefore be stated that for these purine nucleosides the hydrolysis reaction proceeds according to mechanism A-1.

4. The acidic hydrolysis of nucleotides.

Information on the relative stability of the glycosyl bond in mononucleotides as compared with respective nucleosides is rather limited, just like mechanistic considerations on the acidic hydrolysis of nucleotides.

TABLE 8

	k, sec^{-1}
2'-deoxyadenosine-5'-phosphate	3.1×10^{-4} (4.3×10^{-4})
adenosine-5'-phosphate	3.8×10^{-7} (3.6×10^{-7})
2'-deoxyguanosine-5'-phosphate	1.8×10^{-4} (8.3×10^{-4})
2'-deoxycytidine-5'-phosphate	2.0×10^{-8} (1.1×10^{-7})

TABLE 9

	10^4k, sec^{-1}
2'-deoxycytidine-5'-phosphate	0.6
2'-deoxycytidine-3',5'-diphosphate	0.28 (3.7)
5-methyl-2'-deoxycytidine-5'-phosphate	0.58
5-methyl-2'-deoxycytidine-3',5'-diphosphate	0.27 (2.9)
thymidine-5'-phosphate	0.23
thymidine-3',5'-diphosphate	0.12 (0.34)

It has been stated that the hydrolysis of deoxyadenosine, adenosine, deoxyguanosine and guanosine nucleotides is accompanied by a preferential breaking of the N-glycosyl bond, and that phosphate groups have only small effects on the rates[9,54]. Below, the results obtained for phosphates are summed up (pH 1, 37°C; respective values for nucleosides are given in parentheses) (TABLE 8)[7,9].

The introduction of the second phosphate group into position 3' causes further slowing down of the reaction (0.4 N H_2SO_4, 100°C; values for nucleosides are given in parentheses) (TABLE 9)[39,40].

The rate of the acidic breaking of the glycosyl bond has been studied for deoxycytidine and thymidine 3' and 5'-phosphates[55]. Tested deoxyribonucleotides hydrolyzed on heating in acid to give a free base (no nucleoside or deamination product was observed). It has been stated that the pH-rate profiles and thermodynamic parameters resemble those of the parent nucleosides. It is likely that the A-1 mechanism observed with the nucleosides[18] also operates at the nucleotide level. In comparison with respective nucleosides, the relative hydrolysis rates for nucleotides are deoxycytidine : deoxycytidine 3'-phosphate : deoxycytidine 5'-phosphate - 1.0 : 0.40 : 0.24 (pH 0.00, 95°C), and in the case of thymidine : thymidine 3'-phosphate : thymidine 5'-phosphate - 1.0 : 0.74 : 0.70 (at H_o = 2.20, 75°C). Thus, the presence of the phosphate group hinders hydrolysis, and the observed variations of hydrolysis rates are probably caused by conformational changes of the sugar ring. Comparative X-ray structural studies of nucleosides and nucleotides indicate that the presence of the phosphate group or a slightly different substituent in the base is sufficient to alter the solid-state conformation of the sugar ring[56]. Most probably these effects take place in solution as well.

The rate of hydrolysis of the glycosyl bond of wyosine 5'-monophosphate is not lower, but almost identical to that of parent nucleoside[27]. The pH-rate profile for 5'-monophosphate resembles that of parent nucleoside. Mechanism A-1 operative for hydrolysis of nucleoside seems to be also the mechanistic pathway for the hydrolysis of respective monophosphate.

5. Conclusion.

The glycosyl bond constitutes a crucial component of nucleic acids, and its stability conditions the correct functioning of living organisms. A detailed knowledge of the factors causing the breaking of these bonds is continually desired. The acidic hydrolysis of nucleosides is still extensively studied and so far we have at our disposal only some information about the kinetics and mechanism of the acidic hydrolysis of hypermodified nucleosides. The problem of acidic hydrolysis of nucleosides and nucleotides is complex: 1/ depending on the conditions neutral, mono- or diprotonated forms of nucleosides are subjected to hydrolysis, 2/ the differences between the rates of hydrolysis of some substrates change according to pH, 3/ detailed study of the reactive individuals afford probable possibilities for a better explanation of the observed quantitative differences in the rate of acidic hydrolysis of various nucleosides, 4/ the mechanistic investigations are still developed to obtain a more holistic picture of the acidic hydrolysis of nucleosides and nucleotides.

On the other hand, the mechanism of the acidic hydrolysis of nucleosides and nucleotides is an interesting methodological problem, since its reconstruction, and the determination of its cognitive significance is important both for a methodologist and a chemist's research practice. With the trend towards a deeper and more penetrating knowledge of chemical motion, it is worthwile to realize, whether the accepted reconstruction of the mechanism of a given change is satisfactory at the present stage of the development of chemistry.

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