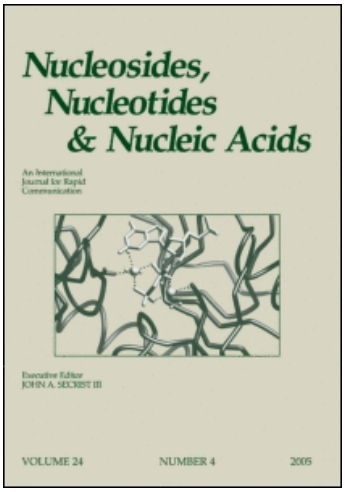

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KINETICS FOR THE ACID-CATALYZED HYDROLYSIS OF PURINE AND CYTOSINE 2'-DEOXY-4'-THIONUCLEOSIDES[1]

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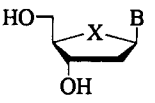
Abstract: Hydrolysis of adenine, guanine and cytosine 2'-deoxy-4'-thionucleosides in aqueous acid has been followed by HPLC. Stability of the compounds and mechanisms of hydrolysis are discussed in comparison with those of the corresponding native 2'-deoxynucleosides.

The 4'-thio analogs of 2'-deoxynucleosides have been extensively investigated[1-3] as potential antiviral agents. Furthermore, since oligonucleotides containing 2'-deoxy-4'-thionucleosides have been shown to form stable duplexes with RNA and to exhibit desired resistance towards some nucleases, they are considered promising compounds for development of antisense oligonucleotide strategies.[4]

Due to the biological applications of 4'-thionucleosides, it appears important to compare also their intrinsic chemical properties to those of their native counterparts. Some differences are expected to exist,[5] because of different nature of oxygen and sulfur. In the present study we have compared the stability of the *N*-glycosidic linkage of the common 2'-deoxynucleosides and their 4'-thio analogs towards acidic hydrolysis. Hydrolytic stability is one of the physico-chemical properties, which may to some extent influence the applicability of the compounds.

Hydrolysis of the nucleoside analogs **1-3** in aqueous acid was followed by analyzing by RP HPLC

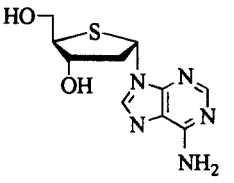


X = S, O

1a: B = cytosine

1b: B = guanine

1c: B = adenine



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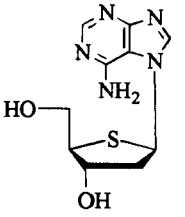

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[1] Dedicated to the memory of Professor Richard T. Walker, 1937 - 1997.

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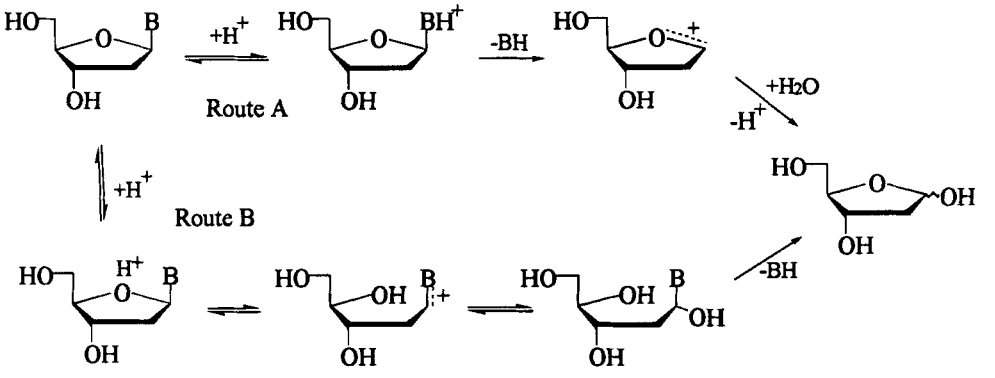
the composition of the samples withdrawn at appropriate time intervals from the reaction solution. Degradation of the purine nucleoside analogs was accompanied by accumulation of a single chromophoric product, the free nucleoside base that was identified by spiking with an authentic sample. With the cytosine derivative **1a**, in contrast, deamination to 4'-thio-2'-deoxyuridine competed with cleavage of the *N*-glycosidic bond (release of cytosine), representing 15 to 20 % of the total hydrolysis both in hydrogen chloride solutions (1.0 and 0.1 M) and in formic and acetic acid buffers (pH 3 to 5). The ratio was determined from accumulation of the two products in the early stages of the reaction. Both reactions are acid-catalyzed at pH > 4 and pH-independent at pH 0 - 4, analogously with the corresponding reactions of cytidine[7] and 2'-deoxycytidine[6]. With 2'-deoxycytidine (**1a**, X = O), however, deamination does not compete with *N*-glycosidic hydrolysis.[6]

Two mechanisms are known to compete in the acid-catalyzed hydrolysis of the *N*-glycosidic bond of 2'-deoxynucleosides (Scheme1).[8] Hydrolysis of purine 2'-deoxynucleosides[9,10] and 2'-deoxycytidine[6,11] follow Route A (Scheme 1), which involves unimolecular rate-limiting departure of a protonated nucleoside base, to leave a cyclic oxocarbenium ion derived from the sugar moiety. No other nucleosidic products or intermediates accumulate during the degradation of these compounds. During hydrolysis of thymidine and 2'-deoxyuridine, by contrast, accumulation of three intermediates, *viz.* the α-anomer of the nucleoside and its *α*- and *β*-pyranoside counterparts, has been observed.[12,13] This is consistent rather with hydrolysis mechanism B of Scheme 1, in which protonation of the 4'-oxygen leads to opening of the C1'-*O*$^{4'}$ bond and formation of a cationic Schiff-base intermediate. Recyclization of the intermediate competes with hydrolytic cleavage of the *N*-glycosidic bond.

As seen from the first-order rate constants reported in Table 1, all the 4'-thionucleosides studied are more stable towards acidic hydrolysis than are the corresponding "natural" 2'-deoxynucleosides. The difference is about 7 fold with the cytosine nucleosides, but ranges from 40 to 70 fold with the purine



SCHEME 1

TABLE 1. First-order Rate Constants for the Hydrolysis of 2'-Deoxynucleosides (dN) and their 4'-Thio Analogs (4'-S-dN) in Aqueous Hydrochloric Acid and in Buffer Solutions.[a]

Compnd	Base	T/K	$c(H^+)$/M	$k_{obs}/10^{-5}$ s^{-1} 4'-S-dN		dN		Ratio $k_{4'O}/k_{4'S}$
1a	Cyt	363.2	1.0	2.77[b]	± 0.08[c]	20.5	± 0.1	7.4
			0.1	2.30[b]	0.03	16.0	0.2	7.0
			pH 3.0[d]	1.44[b]	0.11			
			pH 4.0[e]	0.92[b]	0.03			
			pH 5.0[f]	0.168[b]	0.017			
1b	Gua	323.2	0.1	4.40	0.16	310	5	70
			0.01	0.858	0.005	31.3	0.5	36
			pH 3.6[g]	0.045	0.004			
1c	Ade	323.2	0.1	5.41	0.14	319[h]		59
			0.01	0.571	0.023	25.1[h]		44
			pH 3.6[g]	0.033	0.004	0.68[h]		21
2	α-Ade	323.2	0.1	8.39	0.25			
			0.01	0.898	0.022			
3	Aden-7-yl	323.2	0.1	297	8			
			0.01	27.7	2.4			

[a]The ionic strength adjusted to 0.1 M with sodium chloride. [b]For total hydrolysis of the starting nucleoside. 15 - 20% of the rate results from deamination and 85 - 80 % from depyrimidination (release of cytosine). [c]Standard deviation of the mean from 10 - 12 samples. [d]Formic acid/sodium formate buffer (0.05/0.01 M). [e]Formic acid/sodium formate buffer (0.01/0.02 M). [f]Acetic acid/sodium acetate buffer (0.01/0.02 M). [g]Formic acid/sodium formate buffer (0.02/0.02 M). [h]From Ref. 10.

derivatives. The rate retardation that 4'-thio-substitution exerts on hydrolysis most likely results from lowered stability of the intermediate cyclic carbocation. The rate of acid-catalyzed hydrolysis of purine nucleosides in general is very largely dependent on the polar nature of the substituents at the sugar moiety: electronegative substituents that destabilize the oxocarbenium ion formed in the rate-limiting stage tend to decrease the rate of hydrolysis (Route A in Scheme 1).[8,14] Accordingly, assuming that 4'-thio substitution does not change the mechanism of hydrolysis of purine 2'-deoxynucleosides, the rate decrease it brings about most likely reflects a lower resonance stabilization of the developing thiocarbenium ion compared to that of the corresponding oxocarbenium ion.

Some evidence for unchanged mechanisms is received from the fact that pH-rate profile of hydrolysis of 2'-deoxy-4'-thiocytosine shows same kind of curvature at pH 4 as earlier reported[6] for 2'-deoxycytidine. Furthermore, since the curvature is known[6] to take place at pH corresponding to the pK_a of the base moiety of the nucleoside, it may be seen that the 4'-thio-substitution does not markedly affect the pre-equilibrium protonation. The pK_a estimated for 2'-deoxy-4'thiocytidine from the rate constants given in Table 1 is 4.0 ± 0.2, whereas the corresponding value reported[6] for 2'-deoxycytidine was 3.70 (the

latter at 95 °C, however). The pK_a for 2'-deoxy-4'-thioadenosine was determined spectrophotometrically. The value obtained, 3.9 ± 0.2 at 25 °C and ionic strength 0.1 M, is comparable to the pK_a 3.80 reported earlier[15] for 2'-deoxyadenosine under the same conditions.

Structural effects observed with various adenine derivatives also suggest that the 4'-thio analogs do behave analogously with their 4'-oxo counterparts. Firstly, the α-anomer **2** of 2'-deoxy-4'- thioadenosine is 1.6 times more reactive than the β-isomer **1c**. An almost equal difference (1.4 fold) has earlier been reported[14] for 2'-deoxyadenosine and its α-anomer. Moreover, 7-(β-**D**-ribofuranosyl)-adenine has been shown[16] to be 33.8 times more reactive than adenosine in acidic hydrolysis (at 25 °C), and even this difference is reproduced in the 4'-thio series, *N*7-glycosylated compound **3** being hydrolyzed 49 times faster than the *N*9-glycosylated isomer **1c** (X = S).

In a recent semiempirical study[17] Buckley and Oppenheimer have compared the relative stabilities of oxo- and thiocarbenium ions. They concluded that there is a substantial resonance participation even of sulfur in the cationic center, and that thiocarbenium ions could, at least in some cases, be even more stable than their oxocarbenium counterparts. However, the authors[17] note that their results do not very reliably predict the reactivities in solution. In fact, the present experimental results appear to be consistent with *lower* stability of the thio analog of the cyclic oxocarbenium ion. Furthermore, our data are in this respect in agreement with the results of Whistler and van Es,[18] who showed, for example, that methanolysis of 2,3,4-tri-*O*-acetyl-α-**D**-xylopyranosyl bromide, known to involve formation of a cyclic oxocarbenium ion, is 40 times faster than that of its xylothiopyranosyl analog. On the other hand, these authors[18] found that methyl α- and β-xylothiopyranosides hydrolyze in aqueous acid 10 to 15 times, respectively, faster than their xylopyranoside anlogs. They suggested,[18] however, that the rate enhancing effect of the thiosubstitution in this case results from enhanced protonation of the exocyclic methoxy group, which overcompensates the rate-retarding effect of the lower stability of the thiocarbenium ion compared to its oxocarbenium counterpart. With the nucleoside analogs used in the present study, as described above, thiosubstitution in the sugar ring does not markedly affect the pre-equilibrium protonation, which takes place at the aromatic base moiety.

EXPERIMENTAL SECTION

Materials. Synthesis of 2'-deoxy-4'-thiocytidine (**1a**, X = S) has been published.[1] Preparation of the adenine derivatives **1c**, **2** and **3**, as well as that of 2'-deoxy-4'-thiouridine will be published elsewhere. 2'-Deoxy-4'-thioguanosine (**1b**, X = S) was a generous gift of Dr. G. W. Koszalka. The "native" 2'-deoxynucleosides and nucleoside bases, used as reference material, were commercial products of Sigma.

Kinetic measurements. The hydrolyses of the nucleoside analogs were followed by an HPLC method described earlier.[19] The reaction solutions (*ca.* 0.1 mM nucleoside) in stoppered tubes were immersed in a thermostatted water bath (± 0.1 K). Aliquots were withdrawn at appropriate intervals, neutralized with sodium acetate and cooled in an ice-bath. The composition of the samples was analyzed by HPLC, using a

Hypersil ODS5 column (4-250 mm, 5 μm) and UV detection. As eluents were used acetic acid/sodium acetate buffers (pH 4.2) containing 0.1 M ammonium chloride and 3 - 7 % (*v/v*) acetonitrile. The first-order rate constants were calculated by applying the integrated first-order rate equation to the diminution of the peak area of the starting nucleoside.

pK_a *Measurements.* The pK_a for 2'-deoxy-4'-thioadenosine (**1c**, X = O) was determined by a spectrophotometric method described earlier.[20] Because of the small difference between the UV-spectra of the protonated and unprotonated species, accuracy of the measurement is only moderate (pK_a = 3.9 ± 0.2).

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