Kinetics for the Acid-catalysed Hydrolysis of *O*-, *S*- and *N*-Bridged 5',8-Cyclonucleosides Related to Adenosine

Alexander Karpeisky,[a] Sergey Zavgorodny,[a] Matti Hotokka,[b] Mikko Oivanen[c] and Harri Lönnberg*[,c]

[a] *Schemiakin Institute of Bioorganic Chemistry, Academy of Sciences of Russia, 117871 Moscow, Russia*

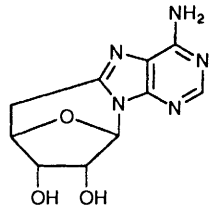
[b] *Department of Physical Chemistry, Åbo Akademi University, FIN-205 00 Turku, Finland*

[c] *Department of Chemistry, University of Turku, FIN-205 00 Turku, Finland*

Kinetics of the acid-catalysed hydrolysis of $O^{5'}$,8-cycloadenosine, $S^{5'}$,8-cyclo-5'-thioadenosine and $N^{5'}$,8-cyclo-5'-amino-5'-deoxyadenosine have been studied. The *S*- and *N*-bridged cyclonucleosides are hydrolysed exclusively by the rupture of the *N*-glycosidic bond, while the *O*-bridged compound undergoes concurrent cleavage of the *N*-glycosidic and 5',8-cyclo linkages, the proportion of the former reaction being markedly increased with increasing temperature. The conformations of the same cyclonucleosides have been elucidated by molecular modelling (SYBYL) and ^{1}H NMR spectroscopy. The effect of the sugar ring puckering on the hydrolytic stability is discussed.

Cyclonucleosides, *i.e.*, nucleoside analogues that contain, besides the *N*-glycosidic bond, another covalent sugar-base linkage, have received interest during the past decade both as useful intermediates of various nucleoside transformation reactions, and as conformationally rigid models of nucleosides. In spite of extensive usage of cyclonucleosides, little is known about the kinetics and mechanisms of their hydrolytic reactions. In fact, the kinetically relevant data are limited to the observations of Lin *et al.*,[1] according to which the *N*-glycosidic bond of the *C*-bridged 5',8-cyclo purine ribonucleosides (**1**, **2**) is hydrolysed from three to nine times more slowly than that of their parent nucleosides (**3a**,**b**), while 3,5'-cyclo-5'-deoxyadenosin-3-ium (**4**) reacts 29 000 times less rapidly than its acyclic analogue, 3-methyladenosinium (**5**). The latter reactivity difference has been attributed to destabilization of the developing oxocarbenium ion intermediate by two parallel influences: (*i*) strong electron-withdrawal by the *N*3-adenyl group at C5', and (*ii*) weakening of the oxygen lone-pair stabilization due to a non-ideal transition-stage geometry.

We now report on the kinetics of the acid-catalysed hydrolysis of *N*-, *O*- and *S*-bridged 5',8-cyclonucleosides related to adenosine (**6a–c**). The conformation of these compounds has been elucidated by molecular mechanics calculations and ^{1}H NMR spectroscopy. Comparison of these data with the hydrolytic stability is aimed at elucidating the susceptibility of the transition-state energy to changes in the initial-state geometry.



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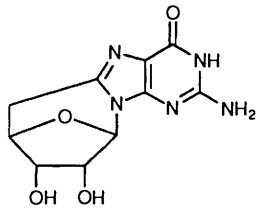

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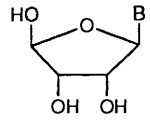
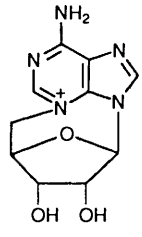


3a; B = adenine
b; B = guanine



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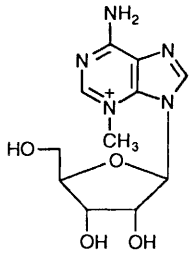

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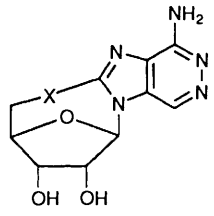

6a; X = O
b; X = S
c; X = NH

Results and Discussion

The hydrolyses of **6a–c** were followed by HPLC and ^{1}H NMR spectroscopy. The results obtained with 5',8-cycloadenosine (**6a**) corroborated the previous observations of Ikehara *et al.*,[2,3] according to which **6a** yields, on treatment with aqueous acid, a mixture of 8-oxo-8,9-dihydro-7*H*-adenosine (**7**), 8-oxo-8,9-dihydro-7*H*-adenine (**8**) and 5-*O*-(8-adenyl)-D-ribose (**9a**). ^{1}H NMR spectroscopic measurements in ^2H_2O solution of deuterioperchloric acid showed that the disappearance of the anomeric and aromatic proton signals of **6a** (H1', s, 6.10; H2, s, 8.22) was initially accompanied by formation of three major products, each exhibiting one signal in both the anomeric and aromatic proton region (Table 1). Comparison with an authentic sample revealed that one of these compounds was **7**. The other two, exhibiting the H1' resonance at 5.26 ($J_{H1',H2'}$ = 4.0 Hz) and 5.11 ($J_{H1',H2'}$ = 1.2 Hz), and the H2 resonance at 8.16, were assigned as the α- and β-forms of **9a**, respectively. Additionally, a weak signal at 7.95 could be detected. This minor product (< 10%), when characterized by EI mass spectroscopy, exhibited a molecular peak at m/z = 169, and on this basis it was tentatively assigned as 5-carboxylamino-4,6-diaminopyrimidine. On prolonged treatment, the H2 signal of the 8-oxoadenine (**8**) appeared at 8.15. HPLC analysis verified that **6a** is decomposed directly to **7** and **9a**, without

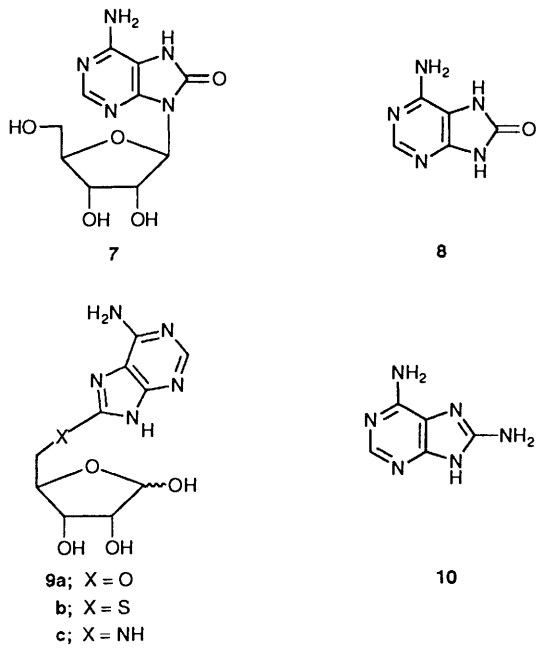

Table 1 Physicochemical data for the *O*-, *S*- and *N*-bridged 5′,8-cyclonucleosides **6a–c** and their hydrolysis products

Compd.	t_R/min		λ_{max}/nm[c]	^{1}H NMR chemical shifts[d]		
	a	*b*		H2	H1′	$J_{H1',H2'}$/Hz
6a	8.3	6.0	263[e]	8.22[f]	6.10	< 1
7	9.5	6.8	271 (sh 260)[g]	8.20[f]	5.76	5.5
8	4.1	3.2	268	8.15[f]		
9a	4.1	3.6	270	8.16[f]	5.26	4.0 (α-form)
				8.16[f]	5.11	1.2 (β-form)
h	1.8	2.1	266	7.95[f]		
6b		6.7	285[i]	8.55[j]	6.22	0
9b		4.4	285	8.51[j]	5.13	4.0 (α-form)
				8.51[j]	4.97	0 (β-form)
6c	6.8		279[k]	8.32[f]	6.25	0
9c	3.4		290	8.38[f]	5.46	3.8 (α-form)
	2.4		290	8.37[f]	5.62	0.8 (β-form)
10	2.8		286	8.40[f]		

[a] On Ultracarb 5 ODS 20 (4.6 × 150 mm, 5 μm), using a 95:5 (v/v) mixture of acetic acid buffer (pH 4.2) and acetonitrile as the eluent. [b] On Hypersil ODS (4.6 × 250 mm, 5 μm), using a 97:3 (v/v) mixture of acetic acid buffer (pH 4.2) and acetonitrile as the eluent. [c] At pH 4.2. [d] As ppm from external Me_4Si. [e] Lit.,[2] 260 nm at pH 1, 261 nm at pH 7. [f] In ^2H_2O at p^{2}H = 1. [g] Lit.,[2] 273 nm at pH 1, 267 nm pH 7. [h] Compound identified tentatively as 5-carboxylamino-4,5-diaminopyrimidine. [i] Lit.,[4] 284 nm at pH 1 and 285.5 nm at pH 7. [j] In [$^2H_6$]dimethyl sulfoxide. [k] Lit.,[5] 279 nm in methanol.

accumulation of any UV-absorbing intermediates (Fig. 1). **9a** is subsequently hydrolysed to **8**, whereas **7** is remarkably stable towards hydrolysis (no decomposition during 24 h at [H$^+$] = 0.1 mol dm^{-3}, T = 363.2 K).

HPLC and ^{1}H NMR spectroscopic analyses of the aliquots withdrawn from solutions of 5′,8-cyclo-5′-thioadenosine (**6b**) in aqueous hydrogen chloride (0.1 mol dm^{-3}) showed that the hydrolysis proceeds exclusively by cleavage of the *N*-glycosidic bond. Table 1 records the physicochemical data for the starting material and the products (**9b**).

When hydrolysis of 5′,8-cyclo-5′-amino-5′-deoxyadenosine (**6c**) was followed by ^{1}H NMR spectroscopy in ^2H_2O solution of deuterioperchloric acid, initial formation of two compounds, assigned as the α- and β-anomers of 5-(8-adenylamino)-5-deoxy-D-ribose (**9c**), was observed. This reaction was followed by much slower release of 8-aminoadenine (**10**). HPLC analysis

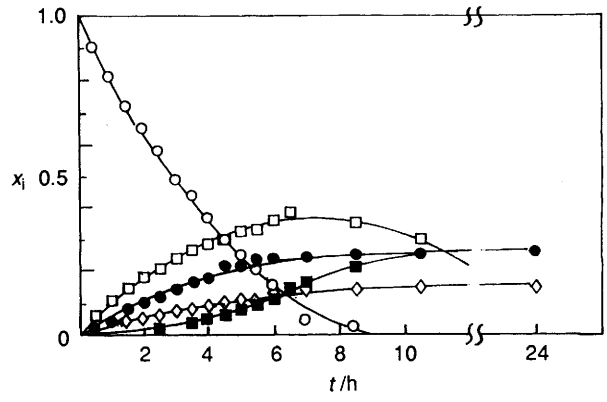

Fig. 1 Time-dependent product distribution for the hydrolysis of $O^{5'}$,8-cycloadenosine (**6a**) in aqueous hydrogen chloride ([H$^+$] = 0.10 mol dm^{-3}) at 363.2 K. Notation: **6a** (○); **7** (●); **8** (■); **9a** (□) and unidentified product (◇).

Table 2 First-order rate constants for the hydrolysis of $O^{5'}$,8-cycloadenosine (**6a**), $S^{5'}$,8-cyclo-5′-thioadenosine (**6b**), and $N^{5'}$,8-cyclo-5′-amino-5′-deoxyadenosine (**6c**)[a]

Compd.	[H$^+$]/mol dm^{-3}	T/K	$k_1/10^{-4}$ s^{-1}	$k_2/10^{-4}$ s^{-1}
6a	1.0	363.2	14 ± 1	6.5 ± 0.7
	1.0	343.2	2.2 ± 0.1	1.7 ± 0.1
	1.0	323.2	0.25 ± 0.01	0.39 ± 0.01
	0.10	363.2	0.73 ± 0.04	0.36 ± 0.02
	0.010[b]	363.2	0.10 ± 0.01	0.051 ± 0.001
6b	0.10	363.2	0.55 ± 0.01	
6c	0.10	363.2	5.8 ± 0.1	

[a] k_1 and k_2 are the first-order rate constants for the cleavage of the *N*-glycosidic and C8–X cyclo linkage, respectively. [b] The ionic strength adjusted to 0.1 mol dm^{-3} with sodium chloride.

also indicated that **10** is formed from **9c** *via* several intermediates, which do not markedly accumulate. Formation of 8-aminoadenosine was not detected.

Table 2 summarizes the rate constants obtained for the hydrolysis of the *N*-glycosidic bond and the C8–X anhydro linkage of the cyclonucleosides studied. As described above, $S^{5'}$,8-cyclo-5′-thioadenosine (**6b**) and $N^{5'}$,8-cyclo-5′-amino-5′-deoxyadenosine (**6c**) are hydrolysed exclusively by the rupture of the *N*-glycosidic bond, while $O^{5'}$,8-cycloadenosine (**6a**) undergoes concurrent cleavage of the *N*-glycosidic and C8–O anhydro linkages. The proportion of the former reaction markedly increases with increasing temperature. This strongly suggests that while the *N*-glycosidic bond of purine nucleosides is cleaved by a unimolecular departure of the protonated base moiety,[6,7] the rate-limiting stage of the hydrolysis of the anhydro linkage is bimolecular, most likely a nucleophilic attack of a water molecule on C8 of the protonated base moiety. This difference in molecularity of the rate-limiting stage is reflected in the entropy of activation, and hence the unimolecular process is favoured at elevated temperatures: $\Delta S^{\ddagger} = -(38 \pm 4)$ J K^{-1} mol^{-1} for the unimolecular cleavage of the *N*-glycosidic bond and $\Delta S^{\ddagger} = -(126 \pm 2)$ J K^{-1} mol^{-1} for the bimolecular cleavage of the anhydro bond at 333.2 K (1 mol dm^{-3} aqueous hydrogen chloride as the reference state).

The first-order rate constant for the hydrolysis of adenosine is 4.64 × 10^{-4} s^{-1} in 0.1 mol dm^{-3} aqueous hydrogen chloride at 363.2 K.[8] Accordingly, the relative hydrolysis rates of the *N*-glycosidic bond of **6a**, **6b** and **6c** compared with that of adenosine are 0.16, 0.12 and 1.2. 8-Methoxy-,[9] 8-methylthio-,[9] and 8-methylamino-[10] substituted adenine nucleosides have, in turn, been shown to be hydrolysed 0.6, 0.4 and 11 times as fast as unsubstituted adenine nucleosides, respectively. In other

Table 3 Torsional angles[a] of *O*-, *S*- and *N*-bridged 5′,8-cyclonucleosides (**6a–c**) calculated by molecular mechanics[11]

Compd.	X	ν_0	ν_1	ν_2	ν_3	ν_4	τ	φ_1	φ_2	φ_3	φ_4	φ_5
4	+42	+16	+8	−26	+37	−33	+96	+129	−31	−86	−23	+92
6a	−111	+29	−8	−15	+33	−39	+40	+113	−17	−90	−74	+43
6b	−113	+21	−6	−10	+23	−28	+44	+117	−12	−100	−73	+45
6c	−122	+26	−7	−13	+30	−35	+60	+115	−15	−94	−58	+58

[a] Given as degrees. X = C4–N9–C1′–O4′, ν_0 = C4′–O4′–C1′–C2′, ν_1 = O4′–C1′–C2′–C3′, ν_2 = C1′–C2′–C3′–C4′, ν_3 = C2′–C3′–C4′–O4′, ν_4 = C3′–C4′–O4′–C1′, τ = X–C5′–C4′–C3′, φ_1 = H1′–C1′–C2′–H2′, φ_2 = H2′–C2′–C3′–H3′, φ_3 = H3′–C3′–C4′–H4′, φ_4 = H4′–C4′–C5′–H5′, φ_5 = H4′–C4′–C5′–H5″.

Table 4 ^{1}H NMR chemical shifts and vicinal ^{1}H, ^{1}H coupling constants of the sugar protons of the *O*-, *S*- and *N*-bridged 5′,8-cyclonucleosides studied[a]

	δ						*J*(H,H)/Hz				
Compd.	H1′	H2′	H3′	H4′	H5′	H5″	1′,2′	2′,3′	3′,4′	4′,5′	4′,5″
6a[b]	6.16	4.62[c]	4.42	4.64[c]	4.61[c]	4.22	< 1	6.1	< 1	2.1	1.2
							(2.2)	(6.2)	(1.0)	(1.7)	(2.7)
6b[d]	6.21	4.70	4.40	4.83	3.22	3.15	1.5	6.3	< 1	2.0	3.4
							(2.5)	(6.4)	(1.4)	(2.2)	(3.9)
6c[b]	6.25	4.40	4.21	4.59	3.60	3.48	1.2	6.0	< 1	2.3	2.3
							(2.3)	(6.3)	(1.1)	(3.8)	(1.5)

[a] As ppm from external Me_4Si. The coupling constants given in parentheses are those calculated according to Haasnoot *et al.*[12] from the torsion angles of the molecular mechanics optimization (see Table 3). [b] In ^2H_2O. [c] Signals of H2′, H4′ and H5′ partially overlap. [d] In [$^2H_6$]DMSO.

words, formation of the 5′,8-*O* and 5′,8-*S* bridges appears to stabilize the *N*-glycosidic bond of the correspondingly substituted adenine nucleosides by a factor of three to four, while the 5′,8-*N* bridge results in a nine-fold stabilization. The rate-retardation is thus comparable to the effect that results from the conversion of purine nucleosides into their 5′,8-cyclo derivatives (**1**, **2**), and is hence much smaller than the effect of 3,5′-bridge formation (**4**).[1] The moderate rate-retardations observed with **1**, **2** and **6a–c** may, at least partially, be attributed to the fact that the adenyl or 8-substituted adenyl group remains bonded to C5′, and hence, as an electronegative substituent, destabilizes the developing glycosyl oxocarbenium ion.

To find out how severely the sugar-ring puckering of **6a–c** differs from that of **4**, the conformations of these compounds were optimized by molecular modelling (SYBYL).[11] The torsion angles obtained are listed in Table 3. The vicinal ^{1}H,^{1}H coupling constants calculated according to Haasnoot *et al.*[12] from the torsion angles of **6a–c** agree fairly well with the experimentally obtained values (Table 4). The consistency is nice for *J*(H2′,H3′) and *J*(H3′,H4′) of all the compounds, and for *J*(H4′,H5′) of **6a** and **6b**. The experimental values of *J*(H1′,H2′) are systematically about 1 Hz smaller than the calculated ones. The differences between the experimental and calculated values of *J*(H4′,H5″) are somewhat larger and unsystematic. One should bear in mind, however, that the experimental coupling constants refer to conformation in an organic solvent and are thus to some extent solvent dependent. The molecular mechanics calculations, in turn, do not take solute–solvent interactions into account. Though the solution conformations may deviate slightly from those obtained by molecular modelling, we believe that the latter approach forms a sound basis for the comparisons of the conformational properties of **6a–c** with those of **1** and **4**. Previously[13] a conformation in which the X–C5 and O4′–4 bonds are nearly eclipsed, has been suggested for 5′,8-cyclonucleosides. However, this type of conformation (τ = 135°) predicts a value of *J*(H4′,H5′) of around 10 Hz, and it also exhibits a higher molecular mechanics energy than the staggered conformations indicated in Table 3.

The high hydrolytic stability of 3,*O*5′-cycloadenosinium (**4**) compared with 3-methyladenosinium (**5**) has previously[1] been attributed to two factors: (*i*) destabilization of the oxocarbenium ion intermediate resulting from the strong electron-withdrawal by the *N*3-adenyl group at C5′, and (*ii*) weakening of the oxygen lone-pair stabilization due to a non-ideal transition-state geometry. The latter argument was based on the observation that the dihedral angle defined by N9–C1′–O4′–(app lone electron pair of O4′) is 167° in **4**, while in **1**, being only moderately more stable than adenosine, the corresponding angle is 180°, and hence optimal for app-assisted bond cleavage. The present results do not lend support for this conclusion. The torsion angle N9–C1′–O4′–C4′ obtained by molecular mechanics for **6a–c** shows a comparable variation, being −89° with **6a**, −101° with **6b** and −93° with **6c**. Most likely the alignment of the app lone electron pair of O4′ with respect to the cleaving *N*-glycosidic bond exhibits a similar variation. However, this variation is not reflected in the hydrolytic stability. It is also worth noting that the torsion angle N9–C1′–O4′–C4′ obtained by SYBYL program for **4** is equal to that obtained for **6b**. Accordingly, it appears quite clear that small differences in the ring-puckering of cyclonucleosides do not markedly affect their hydrolytic stability. The large reactivity difference between **4** and **5** remains to be explained. The inductive destabilization of the oxocarbenium ion intermediate may well be more marked with **4** than with **6a–c**. However, the possible influence of the cyclonucleoside formation on the electronic properties of the base moiety of **5** should also be considered, since the exceptional hydrolytic instability of *N*3-alkylated purine nucleosides has been shown to be of electronic origin.[14]

Experimental

Materials.—*O*5′,8-Cycloadenosine (**6a**),[15] *S*5′,8-cyclo-5′-thioadenosine (**6b**),[4] and *N*5′,8-cyclo-5′-amino-5′-deoxyadenosine (**6c**)[5] were prepared as described previously. Tables 1 and 4 record the physicochemical data for the products obtained.

Kinetic Measurements.—Reactions were followed by the HPLC technique described previously.[16] The initial substrate concentration was 5×10^{-4} mol dm^{-3}. The compositions of the

aliquots withdrawn at suitable intervals were analysed by HPLC on either a Ultracarb 5 ODS 20 (4.6 × 150 mm, 5 μm) or Hypersil ODS (4.6 × 250 mm, 5 μm) column, using a mixture of acetic acid buffer (pH 4.2) and acetonitrile (with Ultracarb 95:5; with Hypersil 97:3; v/v) as the eluent. First-order rate constants for the disappearance of the starting material were calculated by the integrated first-order rate equation. When more than one product was initially formed, this rate constant was bisected to the rate constants of parallel reactions on the basis of the concentration ratio of the products observed by ^{1}H NMR spectroscopy.

^{1}H *NMR Spectroscopic Measurements.*—The ^{1}H NMR spectra were recorded on a JEOL GX-400 spectrometer. The irradiation technique starting from the anomeric proton was applied to assign the signals of the sugar protons.

Molecular Modelling.—The structures of **4** and **6a–c** were determined by the molecular modelling program SYBYL.[11] All the computations were performed by using the purely empirical molecular mechanics method with the standard Tripos parametrization.[17] The default minimizer, MAXMIN2, provided in the SYBYL program was used. This is a hybrid which employs the simplex method for strongly distorted structures with large forces acting on the atoms, and switches to the Powell method, *i.e.*, a method using the numerical first derivatives, when the forces are below a given threshold.

Acknowledgements

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