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ACIDIC HYDROLYSIS OF 6-SUBSTITUTED 9-(2-DEOXY-β-D-*erythro*-PENTOFURANOSYL)PURINES AND THEIR 9-(1-ALKOXYETHYL) COUNTERPARTS: KINETICS AND MECHANISM.[1]

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Abstract: The rate constants for the hydrolysis of several 6-substituted 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purines and 9-(1-alkoxyethyl)purines have been measured at different concentrations of oxonium ion. The effects that varying the polar nature of the alkoxy group exerts on the hydrolysis of unsubstituted 9-(1-alkoxyethyl)purines are interpreted to indicate that the reaction proceeds by a rate-limiting departure of the protonated base moiety with a concomitant formation of an alkoxyethyl oxocarbenium ion. The same mechanism is applied to the hydrolysis of 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purines by comparing the influences that 6-substituents have on the reactivity of these compounds and their 9-(1-alkoxyethyl) counterparts. No sign of anomerization was detected, when the hydrolysis of 2′-deoxyadenosine was followed by ^{1}H NMR spectroscopy.

INTRODUCTION

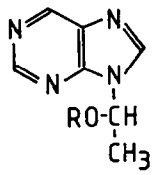
The acidic hydrolysis of purine ribonucleosides has been repeatedly suggested to proceed by a rate-limiting heterolysis of the N-protonated substrate to a ribofuranosyl oxocarbenium ion and free purine base. The experimental evidence presented to support this mechanism includes linear pH-rate profiles,[2-7] lack of anomerization,[8] secondary deuterium isotope effects (1′-d) ranging from 1.18 to 1.23,[6] entropies of activation near zero or positive,[4,5,9] pH-independent hydrolysis of 1,7-dimethylguanosine,[2] insensitivity of the hydrolysis rate to the polar nature of 6-substituents,[7] rate-acceleration by bulky 8-substituents,[8] and enhanced reactivity of *cis*-1′,2′-nucleosides.[10] The hydrolysis of purine 2′-deoxyribonucleosides has been less extensively studied. The experimental data are limited to positive entropies of activation,[2,4,5] almost linear pH-rate profiles,[2,4,5] and the fact that 8-bromo substituent is with 2′-deoxyadenosine only slightly less rate-accelerating than with adenosine.[5] The markedly higher reactivity of 2′-deoxyribonucleosides compared to ribonucleosides has been accounted for by stabilization of the oxocarbenium ion intermediate due to the removal of the electronegative 2′-hydroxyl group.[2,5,10] However, the generality of the mechanism established for the hydrolysis of purine ribonucleosides still needs verification, since the 2′-deoxyriboside of 7-deazaadenine,[11] 2′-deoxyuridine[12] and thymidine[12] have been shown to anomerize in aqueous acid concurrent with their hydrolyses.

The aim of the present paper is to give additional evidence for the rate-limiting oxocarbenium ion formation in the hydrolysis of purine 2′-deoxyribonucleosides. This reaction is of considerable importance, because it is one of the major side re-

actions encountered in the chemical synthesis of oligodeoxyribonucleotides. A knowledge of the reaction mechanism and the structure-reactivity correlations would help to understand the influences of various protecting groups on the stability of the N-glycosidic bond. The problem was approached by comparing the structural effects in the hydrolyses of 6-substituted 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purines and their acyclic counterparts to those observed[13] with acyclic acetals, known to be hydrolyzed via oxocarbenium ions.[14]

RESULTS AND DISCUSSION

Fig. 1 shows the first-order rate constants, k(obs.), for the hydrolysis of some 9-(1-alkoxyethyl)purines (1a-1d) at different concentrations of oxonium ion. The



1a: R = $CH(CH_3)_2$
1b: R = CH_2CH_3
1c: R = CH_3
1d: R = CH_2CH_2Cl

rate of hydrolysis is markedly reduced as the oxygen bonded alkyl group, R, becomes more electronegative, the dependence of k(obs.) on $[H^+]$ being linear with all the compounds studied. These observations are consistent with a mechanism involving a rapid initial protonation of the purine ring and a subsequent rate-limiting formation of an alkoxyethyl oxocarbenium ion (Route A in Scheme 1). The increasing electronegativity of group R only slightly retards the pre-equilibrium protonation, as seen from Table 1, but the developing oxocarbenium ion may be expected to undergo a considerable destabilization, and hence the reaction is retarded. For comparison, the acid-catalyzed hydrolysis of acyclic acetals is rather susceptible to the polar nature of the alkyl group, R^1, which remains bonded to the oxocarbenium ion intermediate (Route C in Scheme 2). The relative rate constants of this partial reaction have been reported to be 22.1, 4.48, 1 and 0.048, when R^1 is isopropyl, ethyl, methyl and 2-chloroethyl, respectively.[13] As seen from Fig. 2, the influence of group R on the decomposition of compounds 1a-1d is nearly the same, which strongly

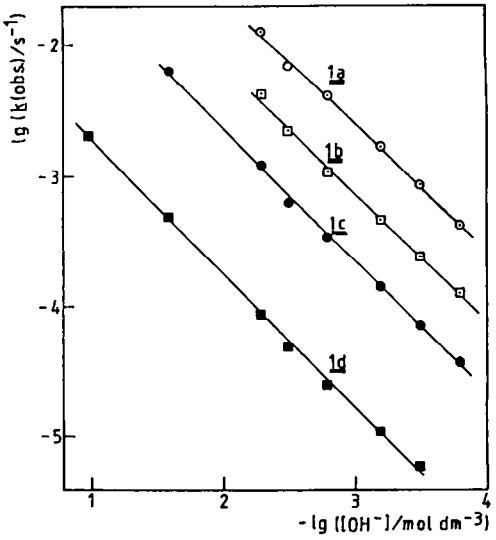
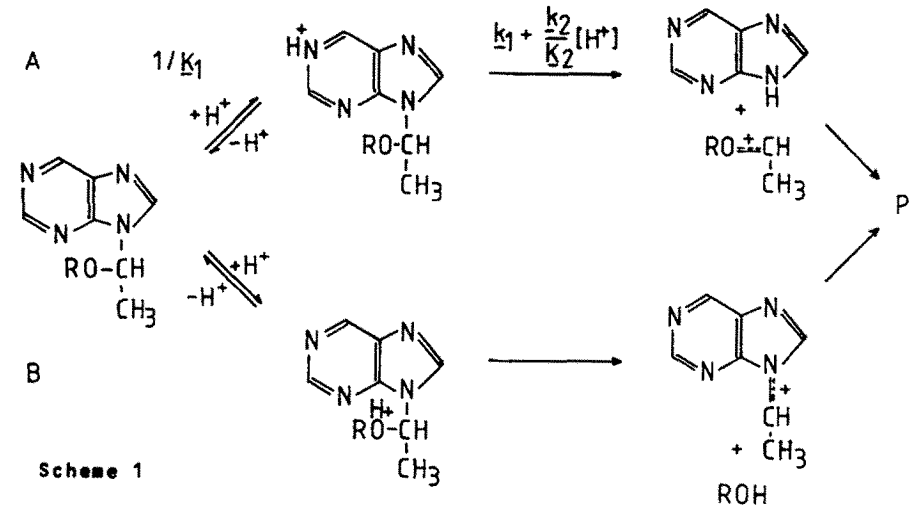
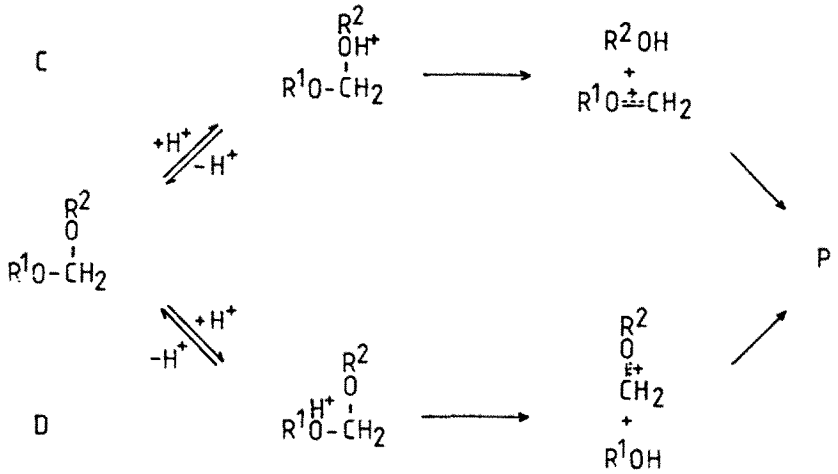


Fig.1: First-order rate constants, k(obs.), for the hydrolysis of 9-(1-alkoxyethyl)-purines (1a-1d) in aqueous acid and buffer solutions at 313.2 K. The ionic strength was adjusted to 0.20 mol dm^{-3} with sodium chloride. The data referring to 1b were taken from Ref. 16.



Scheme 1



Scheme 2

suggests that both reactions proceed via very similar oxocarbenium ions. The slightly lower susceptibility in the hydrolysis of 9-(1-alkoxyethyl)purines may result from the fact that the distance between the oxygen bonded alkyl group and the site of pro-

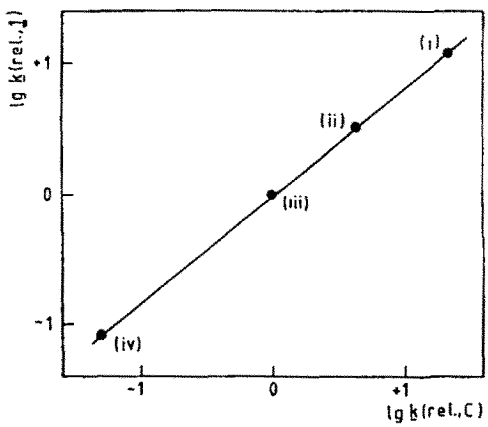


Fig.2: Comparison of structural effects in the acidic hydrolysis of 9-(1-alkoxyethyl)purines and reaction C of acyclic acetals[13] (see Scheme 2). The relative rate constants refer to $[H^+] = 10^{-3}$ mol dm^{-3}. Notation: (i) isopropyl, (ii) ethyl, (iii) methyl and (iv) 2-chloroethyl derivatives.

Table 1: Rate and equilibrium constants for the partial reactions involved in the acidic hydrolysis of 6-substituted 9-(2-deoxy-β-D-*erythro*-pentofuranosyl)purines (3a-3d) and their acyclic analogues (1a-1d, 2a-2d).[a]

Compd	T/K	$-\lg(K_1/\text{mol dm}^{-3})$[b]	$k_1K_1^{-1}/\text{dm}^3\ \text{mol}^{-1}\ \text{s}^{-1}$	$k_2K_2^{-1}/\text{dm}^3\ \text{mol}^{-1}\ \text{s}^{-1}$
1a	313.2	2.19	2.6	2.0
1b	313.2	2.12	0.72	0.78
1c	313.2	2.06	0.21	0.25
1d	313.2	2.09	0.016	0.020
2a	313.2	3.84	0.10	0.14
2b	313.2	3.71	0.20	0.19
2c	313.2	2.80	0.43	0.34
2d	313.2	1.21	0.33	0.35
3a	323.2	3.50	0.017	0.026
3b	323.2	3.67[c]	0.027	0.025
3c	323.2	1.94	0.16	0.13
3d	323.2	1.30	0.067	0.063

[a] k_1/K_1 and k_2/K_2 defined in Scheme 1. For the experimental conditions see the legends of Fig. 1, 3 and 4. [b] Determined spectrophotometrically at 293.2 K and extrapolated to the temperatures indicated by assuming that the dependence of K_1 on T is similar to that observed with 1d. For the latter compound the values of $-\lg(K_1/\text{mol dm}^{-3})$ were 2.19, 2.15, 2.13 and 2.09 at 283.2, 293.2, 303.2 and 313.2 K, respectively. [c] Taken from Ref. 2.

tonation is with 1a-1d larger than with acetals. As shown previously, the most basic nitrogen atom in 9-substituted purines is N1.[15]

If the alkoxy group, OR, would depart in the rate-limiting stage, as depicted by Route B in Scheme 1, k(obs.) could be expected to be relatively insensitive to the polar nature of R. Electron-withdrawal by R, for example, would retard the pre-equilibrium protonation, but simultaneously the rupture of the CO bond would be facilitated. In acetal hydrolysis these two influences almost cancel each other, the relative rate constants of reaction D (Scheme 2) being 2.27, 1.21, 1 and 1.96, when R^1 is isopropyl, ethyl, methyl and 2-chloroethyl, respectively.[13] The decomposition of the cationic Schiff base cannot constitute the rate-limiting step for the disappearance of the starting material, because the departure of the alkoxy group, OR, must be virtually irreversible in a dilute aqueous solution.

We have shown previously[7] that k(obs.) for reaction A may be expressed by eqn. (1), where k_1 and k_2 are the rate constants for the unimolecular heterolyses of the mono- and di-protonated substrate, and K_1 and K_2 are the acidity constants of the

$$k(\text{obs.}) = \frac{k_1K_2[H^+] + k_2[H^+]^2}{K_1K_2 + [H^+](K_2 + [H^+])} \qquad (1)$$

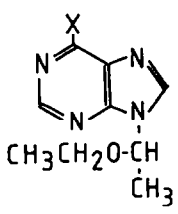
same species. When $[H^+] \leq 20K_1$, $[H^+]$ is negligible compared to K_2, and eqn. (1) can be replaced by eqn. (2). Substitution of the spectrophotometrically measured

$$k(\text{obs.}) \cong \frac{k_1[H^+] + \frac{k_2}{K_2}[H^+]^2}{K_1 + [H^+]} \qquad (2)$$

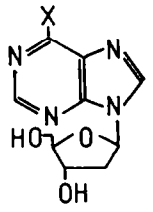
values of K_1 (Table 1) into the latter equation enables the determination of k_1/K_1 and k_2/K_2 by the method of least-squares. The results obtained are listed in Table 1. With each compound (1a-1d) studied the values of these quantities are of the

same order of magnitude. In fact, this is equivalent to the observed linearity of the pH-rate profiles. Since the consecutive acidity constants of protonated purines are known to differ by a factor of 10^4,[18] this means that the diprotonated substrate is heterolyzed 10^4 times more readily than the monoprotonated one.

The rate constants, k(obs.), for the hydrolyses of 6-substituted 9-(1-alkoxyethyl)purines (2a-2e) and 9-(2-deoxy-β-D-erythro-pentofuranosyl)purines (3a-3e) are also linearly related to the concentration of oxonium ion over the whole acidity range studied (Fig. 3 and 4). With both series of compounds the influence of



2a: X = $N(CH_3)_2$
2b: X = NH_2
2c: X = CH_3
2d: X = OCH_3
2e: X = Cl



3a: X = $N(CH_3)_2$
3b: X = NH_2
3c: X = H
3d: X = OCH_3
3e: X = Cl

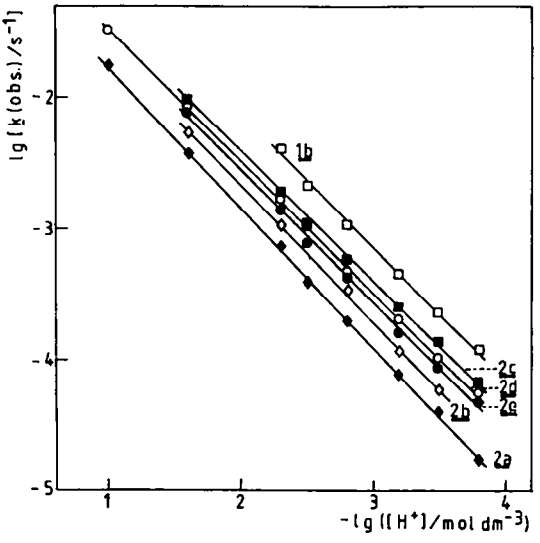


Fig.3: First-order rate constants, k(obs.), for the hydrolysis of 6-substituted 9-(1-ethoxyethyl)purines (2a-2e) in aqueous acid and buffer solutions at 313.2 K. The ionic strength was adjusted to 0.20 mol dm^{-3} with sodium chloride. The data referring to 2b were taken from Ref. 17.

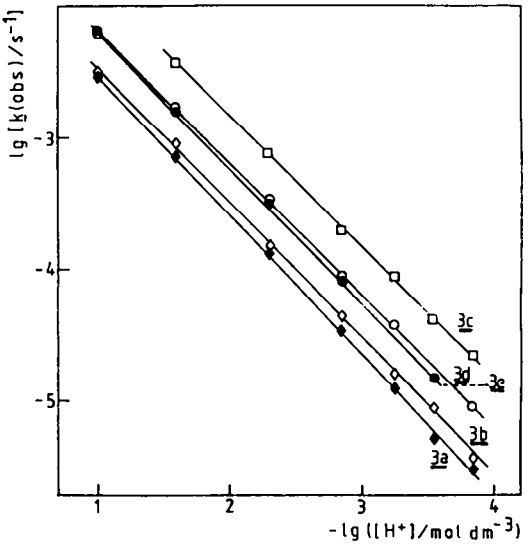


Fig.4: First-order rate constants, k(obs.), for the hydrolysis of 6-substituted 9-(2-deoxy-β-D-erythro-pentofuranosyl)purines (3a-3e) in aqueous acid and buffer solutions at 323.2 K. The ionic strength was adjusted to 0.10 mol dm^{-3} with sodium chloride.

the 6-substituent on k(obs.) is only a moderate one, the electropositive and electronegative groups being both rate-retarding. This is consistent with mechanism A. Increasing electronegativity, for example, markedly retards the pre-equilibrium protonation, as can be seen from the values of K_1 given in Table 1. Simultaneously the purine moiety, however, becomes a better leaving group. In other words, the influences on the initial protonation and rate-limiting heterolysis are opposite, and may largely cancel each other. For comparison, the hydrolysis rate of aryl aldofuranosides, reacting via glycofuranosyl oxocarbenium ions, is virtually independent of the nature of polar substituents in the aglycon moiety.[19] Since different substituents at C6 exert almost identical effects on the reactivities of 9-(2-deoxy-β-D-erythro-pentofuranosyl)purines and their acyclic counterparts (Fig.

5), it appears reasonable to assume that the mechanism suggested for 9-(1-alkoxyethyl)purines (1a-1d) may also be applied to the hydrolysis of 2'-deoxyribonucleosides. It should also be noted that very similar structural effects have earlier been observed in the acidic hydrolysis of 6-substituted purine ribonucleosides.[7] The partial rate constants obtained by eqn. (2) for the decomposition

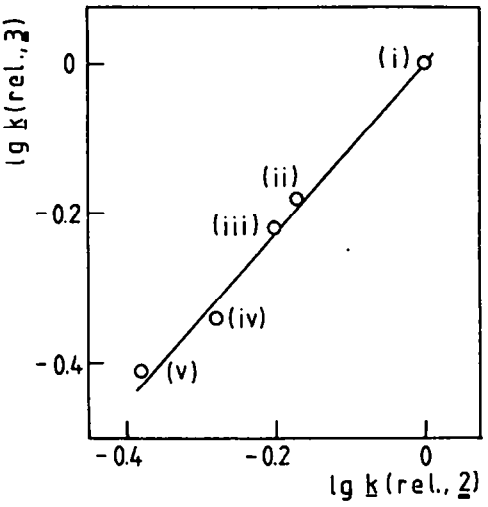


Fig.5: Comparison of structural effects in the acidic hydrolyses of 6-substituted 9-(2-deoxy-β-D-erythro-pentofuranosyl)-purines and 9-(1-ethoxyethyl)purines. The relative rate constants refer to $[H^+] = 10^{-3}$ mol dm^{-3}. Notation: (i) unsubstituted, (ii) 6-methoxy, (iii) 6-chloro, (iv) 6-amino and (v) 6-dimethylamino derivatives.

of compounds 2 and 3 are collected in Table 1. As with compounds 1, the values of k_1/K_1 and k_2/K_2 are of the same order of magnitude, indicating that the influences of the 6-substituents on the reactions proceeding via mono- and di-cations are similar. In consistence with mechanism A, no sign of anomerization was observed, when the hydrolysis of 2'-deoxyadenosine (3b) was followed by ^{1}H NMR spectroscopy (D_2O, $[D^+] = 0.02$ mol dm^{-3}). The only signal detected in the anomeric proton region (6.0-7.5 ppm from DSS) during the whole kinetic run was the anomeric proton triplet of the starting material at 6.69.

The previous finding,[20] according to which N^6-benzoyl-2'-deoxyadenosine is hydrolyzed in a mixture of dioxan and aqueous hydrogen chloride (1:1 v/v, $[H^+] = 0.2$ mol dm^{-3}, T = 293 K) about 8 times more rapidly than 3b, is difficult to explain on the bases of the preceding discussion. Our preliminary kinetic measurements confirm that N^6-benzoylation of 3b really increases the rate of hydrolysis by one order of magnitude at $[H^+] < 0.01$ mol dm^{-3}. However, at $[H^+] > 0.2$ mol dm^{-3} the hydrolysis is accelerated by only a factor of 2. In other words, the exceptionally large acceleration is observed under conditions where the reaction via the monocation prevails. One might tentatively assume that the predominant monocation of N^6-benzoyl-2'-deoxyadenosine is N7 protonated, and not N1 protonated as with 3b. It should be noted that at present this explanation is only a hypothetical one.

EXPERIMENTAL

Materials. The preparation of 9-(1-alkoxyethyl)purines[16,21] (1a-1d) and 6-substituted 9-(1-ethoxyethyl)purines[17,22,23] (2a-2e) has been described earlier. 2'-Deoxyadenosine (3b) and its N^6-benzoyl derivative were commercial products of Sigma Chemical Company, and they were employed as received. The other 9-(2-deoxy-β-D-erythro-pentofuranosyl)purines (3a, 3c-3e) used in kinetic measurements were synthesized as follows.

6-Chloro-9-(2-deoxy-β-D-erythro-pentofuranosyl)purine (3a). 6-Chloro-9-(2-

deoxy-3,5-di-O-p-toluoyl-β-D-erythro-pentofuranosyl)purine (4, 0.2 g, 0.04 mmol), prepared from 2-deoxy-3,5-di-O-toluoyl-D-erythro-pentofuranosylchloride[24] and 6-chloropurine according to Kazimierczuk et al.,[25] was hydrolyzed for 70 min at room temperature in aqueous sodium hydroxide (200 cm^3, 0.01 mol dm^{-3}) containing 30 % (v/v) methanol. The solution was neutralized with acetic acid and evaporated to dryness under reduced pressure. The deprotected product was isolated by HPLC on a Spherisorb RP-18 column (8x250 mm, 5 μm) using a mixture of water and acetonitrile (94/6, v/v) as eluant. The compound obtained was chromatographically homogenous (LiChrosorb RP-18, 10 μm) and exhibited ^1H NMR chemical shifts and UV absorption maxima identical with those reported.[26]

N^6,N^6-Dimethyl-2′-deoxyadenosine (3a). To a solution of 4 (1.52 g, 3 mmol) in dioxan (5 cm^3) 50 cm^3 of aqueous dimethylamine (40 %) was added and the mixture was stirred overnight. The solvent was removed under reduced pressure and the product isolated by column chromatography on silica gel (Kieselgel G, Merck). 3a obtained (81 %) exhibited ^1H NMR chemical shifts and UV absorption maxima identical with those reported.[27]

9-(2-Deoxy-β-D-erythro-pentofuranosyl)-6-methoxypurine (3d). To a solution of 4 (1.01 g, 2 mmol) in dry methanol (20 cm^3) 20 mmol (1.08 g) of sodium methoxide was added. After 24 h at room temperature the solution was neutralized with concentrated aqueous hydrogen chloride at - 5 °C. 5 cm^3 of pyridine was added and the solvent was removed under reduced pressure. 3d isolated by column chromatography on silica gel exhibited ^1H NMR signals (DMSO-d_6) at δ 8.79 (s, 1H, H8), 8.54 (s, 1H, H2), 6.44 (t, J = 7.1 Hz, 1H, H1′), 4.45 (m, 1H, H3′), 4.10 (s, 3H, OCH_3), 3.89 (m, 1H, H4′), 3.56 (m, 2H, H5′), 2.83 - 2.22 (m, 2H, H2′). The UV absorption maxima in methanol were 250 nm (pH 2), 248 nm (pH 7) and 248 nm (pH 13). The yield was 72 %.

9-(2-Deoxy-β-D-erythro-pentofuranosyl)purine (3c). 3c was prepared from 2′-deoxyadenosine via successive acetylation, reductive deamination and deacetylation, as described by Nair and Chamberlein.[28] The product obtained (49 %) exhibited ^1H NMR chemical shifts and UV absorption maxima identical with those reported.[28]

Kinetic measurements. The hydrolyses were carried out in stoppered bottles immersed in a water-bath, the temperature of which was kept constant within 0.1 K. The aliquots (0.5 cm^3) withdrawn at suitable intervals were neutralized immediately with aqueous sodium hydroxide, and their compositions were determined by HPLC (Varian 5000 equipped with a UV-100 detector) on a reversed phase column (LiChrosorb RP-18, 4x250 mm, 10 μm). A mixture of acetonitrile and acetic acid buffer (0.025 mol dm^{-3}, pH 4.3) was employed as an eluant. The initial substrate concentration was of the order of 10^{-3} mol dm^{-3}. Aqueous hydrogen chloride and acetic and formic acid buffers were used as reaction solutions. The ionic strength was adjusted with sodium chloride. The oxonium ion concentrations of the buffers were calculated on the bases of the data reported in literature.[29-31] The first-order rate constants were calculated from the peak heights of the starting materials via the integrated first-order rate-law.

Acidity constants. The acidity constants for the monocations of the compounds investigated were obtained spectrophotometrically (Unicam 1700 spectrometer) as described earlier.[16]

^1H NMR measurements. The progress of the hydrolysis of 3b was followed in acidic D_2O at 323 K by recording the spectrum of the reaction solution at suitable intervals on a Jeol GX-400 spectrometer. The initial substrate concentration was about 0.05 mol dm^{-3} and the equilibrium concentration of oxonium ion about 0.02 mol dm^{-3}.

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REFERENCES

1 Part XXI of the series "Mechanisms for the Solvolytic Decompositions of Nucleoside Analogues".
2 J. A. Zoltewicz, D. F. Clark, T. W. Sharpless and G. Grahe, J. Am. Chem. Soc. 92, 1741 (1970).
3 J. A. Zoltewicz and D. F. Clark, J. Org. Chem. 37, 1193 (1972).
4 L. Hevesi, E. Wolfson-Davidson, J. B. Nagy, O. B. Nagy and A. Bruylants, J. Am. Chem. Soc. 94, 4715 (1972).
5 E. R. Garrett and P. J. Mehta, J. Am. Chem. Soc. 94, 8532 (1972).
6 R. Romero, R. Stein, H. G. Bull and E. H. Cordes, J. Am. Chem. Soc. 100, 7620 (1978).
7 H. Lönnberg and P. Lehikoinen, Nucleic Acids Res. 10, 4339 (1982).
8 F. Jordan and H. Niv, Nucleic Acids Res. 4, 697 (1977).
9 R. P. Panzica, R. J. Rousseau, R. K. Robins and L. B. Townsend, J. Am. Chem. Soc. 94, 4708 (1972).
10 J. L. York, J. Org. Chem. 46, 1271 (1981).
11 F. Seela, S. Menkhoff and S. Behrendt, J. Chem. Soc. Perkin Trans. II 525 (1986).
12 J. Cadet and R. Teoule, J. Am. Chem. Soc. 96, 6517 (1974).
13 P. Salomaa, Ann. Acad. Sci. Fenn. Ser A II 103, 1 (1961).
14 E. H. Cordes and H. G. Bull, Chem. Rev. 74, 581 (1974).
15 N. C. Gonnella and J. D. Roberts, J. Am. Chem. Soc. 104, 3162 (1982).
16 H. Lönnberg, Acta Chem. Scand. A 34, 703 (1980).
17 H. Lönnberg, Acta Chem. Scand. A 35, 317 (1981).
18 R. L. Benoit and M. Frechette, Can. J. Chem. 63, 3053 (1985).
19 H. Lönnberg, A. Kankaanperä and K. Haapakka, Carbohydr. Res. 56, 277 (1977).
20 C. B. Reese, Tetrahedron 34, 3143 (1978).
21 H. Lönnberg, J. Lukkari and P. Lehikoinen, Acta Chem. Scand. B 38, 573 (1984).
22 H. Lönnberg, R. Käppi and E. Heikkinen, Acta Chem. Scand. B 35, 589 (1981).
23 H. Lönnberg, P. Lehikoinen and K. Neuvonen, Acta Chem. Scand. B 36, 707 (1982).
24 M. Hoffer, Chem. Ber. 93, 2777 (1960).
25 Z. Kazimierczuk, H. B. Cottam, G. R. Revankar and R. K. Robins, J. Am. Chem. Soc. 106, 6379 (1984).
26 M. J. Robins and G. L. Basom, Nucleic Acid Chem. 2, 601 (1978).
27 T. Ueda, Y. Nomoto and A. Matsuda, Chem. Pharm. Bull. 33, 3263 (1985).
28 V. Nair and S. D. Chamberlein, Synthesis 401 (1984).
29 H. S. Harned and N. D. Embree, J. Am. Chem. Soc. 56, 1042 (1934).
30 H. S. Harned and B. B. Owen, J. Am. Chem. Soc. 52, 5079 (1930).
31 H. S. Harned and R. W. Ehlers, J. Am. Chem. Soc. 55, 652 (1933).