*Short Communication*

# pH-Independent Depurination of 7-Alkylguanosines and their 5′-Monophosphates

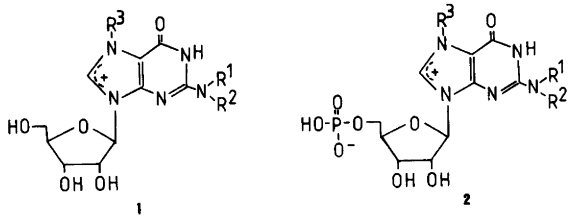
Marjo Lahti,[a] Harri Santa,[a] Edward Darzynkiewicz[b] and Harri Lönnberg[a*]

[a]Department of Chemistry, University of Turku, SF-20500 Turku, Finland and [b]Department of Biophysics, University of Warsaw, 02-089 Warsaw, Poland

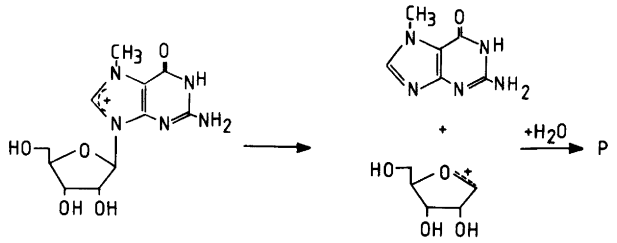
Lahti, M., Santa, H., Darzynkiewicz, E. and Lönnberg, H., 1990. pH-Independent Depurination of 7-Alkylguanosines and their 5′-Monophosphates. – Acta Chem. Scand. 44: 636–638.

Purine nucleoside phosphorylase (EC 2.4.2.1), which catalyzes the phosphorolytic cleavage of the *N*-glycosidic bond of purine nucleosides, is an important target for antitumor and antiviral agents.[1] 7-Alkylguanosines have recently been shown to be good substrates for this enzyme, and the mechanism of phosphorolysis has been elucidated by considering the influence of various 7-substituents on the kinetic parameters ($K_m$, $V_{max}$) in relation to their steric and electronic properties.[2] In the present communication, kinetic data for spontaneous cleavage of *N*-glycosidic bonds of the same nucleosides and their 5′-monophosphates are reported. The data throw light on the structural effect observed for the enzymatic phosphorolysis. Furthermore, spontaneous depurination of 7-alkylguanosines is of interest in view of the fact that the 5′-terminal nuclesoide in eukaryotic mRNAs is 7-methylguanosine.[3]

Fig. 1 shows the pH–rate profile for hydrolytic decomposition of 7-methylguanosine, **1** ($R^1 = R^2 = H$, $R^3 = CH_3$).



The starting material undergoes hydronium-ion-catalyzed depurination at pH < 2,[4] and opening of the imidazole ring at pH > 6.[5] Between these two pH values the decomposition proceeds by uncatalyzed depurination of monocationic 7-methylguanosine, the predominant ionic form in this pH range, and the reaction rate is thus pH-independent (Scheme 1). This reaction mimics the hydrolysis of guanosine at low hydronium ion concentrations, where breakdown of the N7 protonated species constitutes the major reaction pathway.[4,6] It is also worth noting that the enzyme-catalyzed phosphorolysis of guanosine has been suggested to proceed via N7 protonation.[7]



*Scheme 1.*

Table 1 records the first-order rate constants obtained for the spontaneous depurination of various 7-substituted and $N^2$,7-disubstituted guanosines and their 5′-monophosphates. The depurination rates of nucleotides are from 20 to 50 % of those of their parent nucleosides. The reasons for this reactivity difference, which is typical for purine

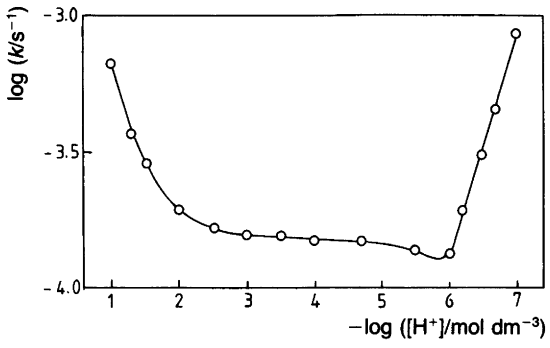



*Fig. 1.* pH–rate profile for the decomposition of 7-methylguanosine at 363.2 K ($I$ = 0.1 mol dm$^{-3}$ with NaCl).

* To whom correspondence should be addressed.

*Table 1.* First-order rate constants ($k/10^{-4}$) for the spontaneous depurination of 7-substituted and $N^2$,7-disubstituted guanosines and their 5′-monophosphates at 363.2 K.[a]

| Substituents[b] | | | | 1 | 2 |
|---|---|---|---|---|---|
| $R^1$ | $R^2$ | $R^3$ | Others | | |
| Hydrogen | Hydrogen | Methyl | | 1.37(5) | 0.327(9) |
| Hydrogen | Hydrogen | Ethyl | | 1.09(2) | 0.219(4) |
| Hydrogen | Hydrogen | Propyl | | | 0.308(7) |
| Hydrogen | Hydrogen | Butyl | | 1.04(3) | 0.417(9) |
| Hydrogen | Hydrogen | Isopropyl | | | 0.144(13) |
| Hydrogen | Hydrogen | Isobutyl | | 1.46(2) | 0.267(13) |
| Hydrogen | Hydrogen | Cyclopentyl | | | 0.290(11) |
| Hydrogen | Hydrogen | Allyl | | 1.89(6) | 0.588(18) |
| Hydrogen | Hydrogen | Benzyl | | 2.62(5) | 0.940(19) |
| Hydrogen | Hydrogen | 1-Phenylethyl | | 1.88(4) | 0.691(11) |
| Hydrogen | Hydrogen | 2-Phenylethyl | | 1.72(3) | 0.453(6) |
| Hydrogen | Hydrogen | 3-Phenylpropyl | | 1.29(3) | 0.271(4) |
| Methyl | Hydrogen | Methyl | | 0.835(9) | 0.212(3) |
| Ethyl | Hydrogen | Methyl | | | 0.195(4) |
| Benzyl | Hydrogen | Methyl | | | 0.234(8) |
| Methyl | Methyl | Methyl | | 0.586(9) | 0.160(3) |
| Hydrogen | Hydrogen | Methyl | 8-Methyl | 1.80(5) | 0.853(22) |

[a]Obtained in an acetic acid/sodium acetate buffer (0.1/0.1 mol $dm^{-3}$). [b]See structures **1** and **2**.

nucleosides and nucleotides, have been discussed previously.[8] The effect of the 7-substituent on the depurination rate is presented in Fig. 2 by a plot of the logarithmic rate constants as a function of polar substituent constants, σ*. In spite of the large scattering of individual points, it is apparent that increasing electronegativity of the 7-substituent considerably destabilizes the *N*-glycosidic bond, the reaction constant being 0.8 ± 0.2 ($r$ = 0.87) with 7-alkylguanosines and 1.2 ± 0.3 ($r$ = 0.82) with their 5′-monophosphates. By contrast, the reaction constant for acid-catalyzed depurination of 7-alkylguanosines, which most likely proceeds by N3 protonation,[4] is only slightly positive (<0.2).[9] Accordingly, electron-withdrrawing groups, which accelerate the heterolytic cleavage of N9-C bond by increasing the positive charge at the imidazole ring, retard almost as efficiently the pre-equilibrium protonation, leaving the observed rate constant practically unchanged. The susceptibility of N3 protonation to the polar nature of the 7-substituent is thus similar to that for N1 protonation, for which a reaction constant of −1.2 has been reported.[9]

The effect of $N^2$-substituents on the rate of spontaneous depurination is considerably weaker than that of 7-substituents. As seen from Fig. 3, a fairly good linear correlation exists between the logarithmic rate constants obtained with $N^2$-substituted 7-methylguanosine 5′-monophosphates and the Σσ* values of their $N^2$-substituents, the reaction constant being 0.31 ± 0.04 ($r$ = 0.97). One might expect that bulky substituents at $N^2$ would increase non-bonded interactions between the base and sugar moieties, and hence result in steric acceleration. However, this does not

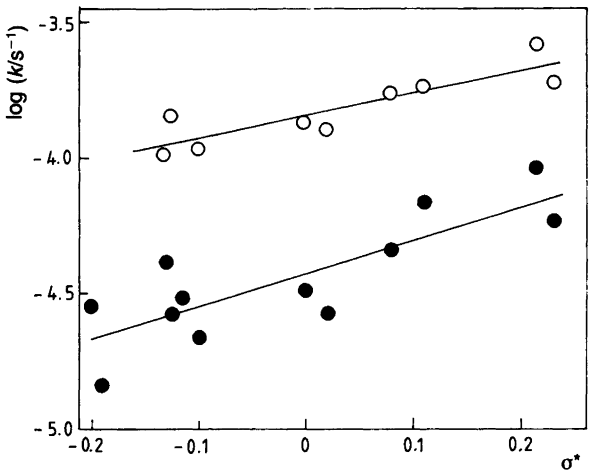



*Fig. 2.* Logarithmic rate constants for spontaneous depurination of 7-alkylguanosines (○) and their 5′-monophosphates (●) plotted against the σ* values of 7-substituents (see Table 1).

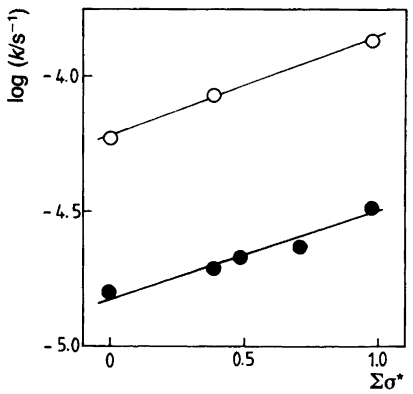



*Fig. 3.* Logarithmic rate constants for spontaneous depurination of $N^2$-substituted 7-methylguanosines (○) and their 5′-monophosphates (●) plotted against the Σσ* values of $N^2$-substituents (see Table 1).

seem to be the case; the point referring to the $N^2$ unsubstituted compound falls on the line that the substituted compounds yield. The data on hydrolysis of $N^2$-substituted 7-methylguanosines are scarce, but consistent with the arguments presented. Accordingly, $N^2$-substituents do not sterically destabilize the *N*-glycosidic bond of either 7-alkylguanine nucleosides or nucleotides. By contrast, 8-substituents appear to bring about a moderate steric acceleration. 7,8-Dimethylguanosine and its 5′-monophosphate are depurinated 1.3 and 2.6 times as fast as their 7-methyl counterparts, although a methyl group may be expected to retard depurination inductively. Comparable steric acceleration has previously been observed in the hydrolysis of 2-substituted benzimidazole nucleosides[10] and their acyclic analogues.[11]

## Experimental

7-Alkylguanosine 5′-monophosphates (**2**) were prepared as described previously,[5,12,13] and converted into the corresponding nucleosides by dephosphorylation with bacterial alkaline phosphatase.[2] First-order rate constants for the disappearance of 7-alkylguanosines and their 5′-monophosphates were calculated via the integrated first-order rate equation. The time-dependent concentrations of the starting materials were obtained by the HPLC method described earlier.[14] Chromatographic separations were carried out under conditions described in Ref. 8.

## References


1. Stoeckler, J. D. In: Glazer, R. I., Ed., *Developments in Cancer Chemotherapy,* CRC Press, Boca Raton 1984, pp. 35–60.
2. Bzowska, A., Kulikowska, E., Darzynkiewicz, E. and Shugar, D. *J. Biol. Chem. 163* (1988) 9212.
3. Rhoads, R. E. *Prog. Mol. Subcell. Biol. 9* (1985) 104.
4. Zoltewicz, J. A., Clark, D. F., Sharpless, T. W. and Grahe, G. *J. Am. Chem. Soc. 92* (1970) 1741.
5. Darzynkiewicz, E., Stepinski, J., Tahara, S. M., Stolarski, R., Ekiel, I., Haber, D., Neuvonen, K., Lehikoinen, P., Labadi, I. and Lönnberg, H. *Nucleosides, Nucleotides 9* (1990). *In press* and references therein.
6. Hevesi, L., Wolfson-Davidson, E., Nagy, J. B., Nagy, O. B. and Bruylants, A. *J. Am. Chem. Soc. 94* (1972) 4715.
7. Bzowska, A., Kulikowska, E. and Shugar, D. *Nucleosides, Nucleotides 8* (1989). *In press.*
8. Oivanen, M., Darzynkiewicz, E. and Lönnberg, H. *Acta Chem. Scand., Ser. B 42* (1988) 250.
9. Muller, N. and Eisenbrand, G. *Chem.-Biol. Interact. 53* (1985) 173.
10. Oivanen, M., Lönnberg, H., Kazimierczuk, Z. and Shugar, D. *Nucleosides, Nucleotides 8* (1989) 133.
11. Lönnberg, H. and Käppi, R. *Tetrahedron 36* (1980) 913.
12. Darzynkiewicz, E., Ekiel, I., Tahara, S. M., Seliger, L. S. and Shatkin, A. J. *Biochemistry 24* (1985) 1701.
13. Darzynkiewicz, E., Stepinski, J., Ekiel, I., Goyer, C., Sonenberg, N., Temeriusz, A., Jin, Y., Sijuwade, T., Haber, D. and Tahara, S. M. *Biochemistry 28* (1989) 4771.
14. Oivanen, M., Lönnberg, H., Zhou, X.-X. and Chattopadhyaya, J. *Tetrahedron 43* (1987) 1133.


Received January 2, 1990.