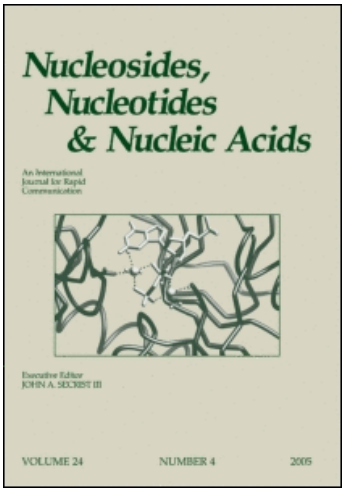

Nucleosides, Nucleotides and Nucleic Acids
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597286

Comparison of Acid- and Enzyme-Catalyzed Cleavage of the Glycosidic Bond of N(7)-Substituted Guanosines

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To cite this Article Bzowska, Agnieszka , Kulikowska, Ewa and Shugar, David(1990) 'Comparison of Acid- and Enzyme-Catalyzed Cleavage of the Glycosidic Bond of N(7)-Substituted Guanosines', Nucleosides, Nucleotides and Nucleic Acids, 9: 3, 439 — 440
To link to this Article: DOI: 10.1080/07328319008045164
URL: http://dx.doi.org/10.1080/07328319008045164

NUCLEOSIDES & NUCLEOTIDES, 9(3), 439-440 (1990)

COMPARISON OF ACID- AND ENZYME-CATALYZED CLEAVAGE OF THE GLYCOSIDIC BOND OF N(7)-SUBSTITUTED GUANOSINES

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Abstract: Kinetic parametrs for enzymatic cleavage of the glycosidic bond (phosphorolysis) of ten N(7)-substituted guanosines were determined, and used to establish a structure-activity relation for the Michaelis constants. Results were compared with those for acid-catalyzed cleavage of the glycosidic bonds, and are consistent with a mechanism for phosphorolysis via protonation of the purine ring N(7).

A study has been made of the effects of various substituents at N(7) of guanosine on susceptibility to acid-catalyzed and phosphorolytic (calf spleen purine nucleoside phosphorylase) cleavage of the glycosidic bond. The electronic properties of such substituents, expressed as Taft electronic constants σ^*,[1] are correlated with the rate constants for acid-catalyzed depurination, which are increased by electron-withdrawing substituents.[2]

Kinetic parametrs have now been determined for enzymatic phosphorolysis (followed spectrophotometrically) of ten N(7)-substituted guanosines,[3,4] methyl (m), ethyl (et), propyl (pr), isopropyl (ipr), butyl (bu), isobutyl (ibu), benzyl (bn), 1-phenylethyl (1phet), 2-phenylethyl (2phet) and carboxymethyl (cm). Correlation were then sought between the Michaelis constants K_m and the electronic, steric and hydrophobic properties of the substituents expressed in terms of the Taft electronic constants σ^*, the Taft steric constants E_s,[1] and the Hansch constants,[5] respectively.

Since K_m for 7-methylguanosine is pH-dependent, the cation with a proton at N(1) being the preferred substrate, relative to the zwitterion,[6] correlations were based on K_m values for the cations, K_m^+, calculated as follows:

$$K_m^+ = K_m(10^{pH - pK_a + 1})^{-1} \qquad (1.1)$$

where K_m was determinrd at pH 7, and pK_a for dissociation of the proton at N(1) was calculated from the linear dependence of pK_a on σ^* of the N(7)-substituent.[2]

The "stepwise variable selection" procedure led to the following equation which best describes the observed values of K_m^+:

$$\log K_m^+ = 0.85(16.01) - 1.02(12.25)\ E_s + 9.55(5.24)(\sigma^*)^2 \qquad (1.2)$$

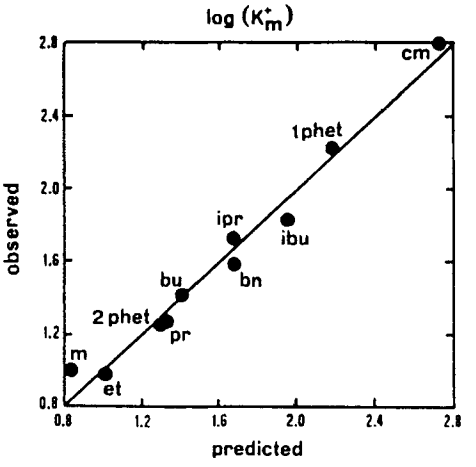

Fig. 1: Plot of experimental values of $\log K_m^+$ for N(7)-substituted guanosines vs values predicted by equation (1.2).

with a correlation coefficient r = 0.989, adjusted for the number of degrees of freedom R = 0.986, with standard deviation s = 0.095 and a Fischer coefficient $F_{2,7}$ = = 155.58. Figures in brackets are ratios of the fitted coefficients to their standard errors.

Note that there is no dependence of K_m^+ on hydrophobic/hydrophilic properties; whereas an increase in steric hindrance, expressed in terms of E_s, leads to an Increase in K_m^+. Appearance of the square of σ^* in the equation (1.2) points to protonation of the imidazole ring of guanosine, during phosphorolysis, since then both electron-withdrawing and electron-donating substituents result in an increase in the value of K_m^+ (effect of deprotonation and protonation, respectively). The overall results suggest that, as for acid-catalyzed hydrolysis of the glycosidic bond,[7] enzymatic phosphorolysis of guanosine proceeds via protonation of the purine ring N(7).

Acknowledgments: We are indebted to Dr. E. Darzynkiewicz for syntheis of N(7)-substituted guanosines; and for support by the Ministry of Higher Education (RP-II 13.1.8), the Polish Cancer Research program (CPBR II.5-109/b) and the Polish Academy of Sciences (CPBR 3.13.1.1.7).

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