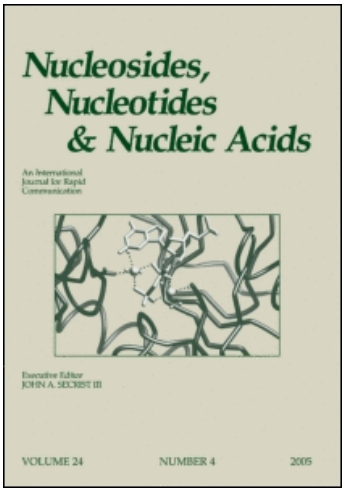

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NMR Studies of the Tautomerism in Pseudoisocytidine

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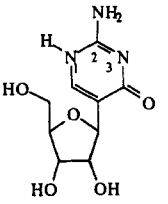
Abstract. The structure of pseudoisocytidine may have two isomers. We would like to designate them as K1 and K3 for N_1H and N_3H, respectively. The authenticity of these two isomers was judged by ^1H NMR. The chemical shift value of N_1H in K1 is found more upfield than N_3H in K3, whereas the chemical shifts of rest protons remain the same. Theoretical calculations show that K1 is less stable than K3 by ca. 9 Kcal/mol in gas phase while a methyl group replaces the furanose moiety. This energy reduces as low as 2 Kcal/mol in solution depending on the polarity of the solvent. Thus, the equilibrium of two tautomers occurs most likely in solution. The ^1H and ^{13}C NMR studies have been carried out in the pH range of 1 to 12. The pKa's of deprotonation of N_1 and N_3 sites are found to be 9.36 and 9.42, for K1 and K3, respectively. On the other hand, the pKa's of protonation of the same sites corresponding to these two isomers are 3.79 and 3.69, respectively. A critical analysis of line broadening of C_2 in K1 and K3 in the pH range of 5 to 7 establishes the proton exchange phenomenon. The exchange rate, catalyzed by both $[H^+]$ and $[OH^-]$, depends on the pH.

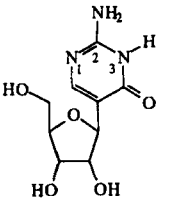
Selected examples of synthetic C-nucleosides that do not occur in nature include pseudoisocytidine. This has two isomeric forms and either one in termed as pseudoisocytidine. For the sake of simplicity we designate them as pseudoisocytidine K1 for the one in which H rests at N_1 position and K3 for the other in which H rests at N_3 position (see figure below).

Theoretical calculations show that K1 is less stable than K3 by ca. 9 Kcal/mol in gas phase (while the furanose moiety is replaced by a methyl group). This energy dramatically reduces in solution depending on the polarity of the solvent and can rest as low as 2 Kcan/mol (see the Table in the previous page).

We have taken a maximum care to trap the higher energy species (K1) instantaneously. The conversion of K1 to K3 in basic medium was obvious but the rate of

K1





K3

		K1		K3			
Level	ε	E	dipole	E	dipole	ΔE	ΔE
	ε_0	a.u.	D	a.u.	D	a.u.	kcal/mol
HF/6-31++G**	1.0	-431.67192	8.31	-431.68706	3.82	0.01514	9.50
HF/6-31++G**(IPCM)// HF/6-31++G**	78.3	-431.70769	11.64	-431.71178	4.80	0.00409	2.57
HF/6-31++G**(SCIPCM)// HF/6-31++G**	78.3	-431.70942	11.76	-431.71393	5.13	0.00451	2.83
HF/6-31++G**(SCIPCM)	78.3	-431.71242	13.20	-431.71513	5.92	0.00271	1.70

conversion could be slowed down to a minimum by treating with methanolic ammonia solution prior to salt formation. By this treatment, we get exclusively K1 isomer.

The authenticity of the two isomers was judged by recording their ^{1}H NMR spectra periodically in varying concentrations. The position of NH in K1 (9.07 ppm) remains static both at higher concentration (ca. 20 mmol) and lower concentration (ca. 4 mmol) over a period of 12 hrs at room temperature. The NH signal of K3 position at 10.92 ppm remains static even after 20 days at room temperature. The authenticity of NH positions in two isomers were further justified by carrying out two experiments pertaining to energy equilibration. In one mixture we took excess of K1 over K3 and found NH signal at 9.40 ppm in the beginning which went to 9.60 ppm within 5 days. Similarly for another mixture in which K3 dominated over K1, the NH signal appeared at 10.45 ppm in the beginning and went to rest 10.17 in 5 days. The upshift of chemical shift is possible only in the presence of high energy isomer i.e. K1 in the solution. We did not get signals for K1 and K3 isomers separately in both the experiments. This fact explains energy exchange between the two isomers.

The ^{1}H and ^{13}C NMR studies have also been carried out in the pH range of 1 to 12. The pKa of deprotonation for NH of K1 is found to be 9.36 and that for K3 is found to be 9.42. The appearance of α–form of pseudoisocytidine below pH 3.58 indicates subsequent ring opening and ring closure in acidic condition. A critical analysis on line

broadening of C_2 in pseudoisocytidine in the pH range of 5 to 7 establishes the proton exchange phenomenon between K1 and K3. The exchange rate catalyzed by both [H^+] and [OH^-]. The detail of this study would be published elsewhere.

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