EFFECT OF THE SOLVENT ON THE ^{13}C NMR SPECTRA OF PYRIMIDINE NUCLEOSIDES

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The ^{13}C NMR spectra of uridine and cytidine in d_6-DMSO, d_7-DMF, d_9-trimethyl phosphate, d_4-methanol, d_5-pyridine, and D_2O were investigated. A linear correlation between the C_1' chemical shifts and the $J_{1'}$ vicinal spin–spin coupling constants of the protons was established. From the experimental data it may be assumed that the chief reason for the effect of the solvent on the $C_{1'2'}$ chemical shift is the different contribution of the α effect of the base as a consequence of a change in the conformational equilibrium of the ribose ring. Deviations from the correlation in aqueous solutions and solutions of cytidine in pyridine are observed as a result of a change in the electron density in the base. The effect of the nature of the solvent on the position of the conformational equilibrium of the base relative to the glycoside bond was demonstrated.

The possibilities of the application of ^{13}C NMR spectroscopy in the investigation of the conformations [1-5] and tautomerism [6] of the components of nucleic acids have been demonstrated, and the effect of the pH, temperature, concentration, substituents, and other factors that are of practical importance in the study of biological systems has been investigated in detail [4, 5]. However, the effect of solvents on the ^{13}C NMR spectra, which is of interest in the organic synthesis of these compounds, has not been studied systematically.

In the present research we investigated the ^{13}C NMR spectra of pyrimidine nucleosides, viz., uridine and cytidine, in a number of solvents that are most often used in organic synthesis. The spectra were interpreted in accordance with [7, 8].

The changes in the chemical shifts in the spectra of the bases of the investigated nucleosides are evidently associated with the effect of the solvent on the distribution of the electron density in the bases [9] as a result of their specific interaction, which is displayed most markedly in protic solvents (Table 1) as a consequence of the formation of hydrogen bonds.

It is well known [2-5] that the conformational transitions in the components of nucleic acids have a substantial effect on the ^{13}C chemical shifts of ribose. A change in the conformation of the base relative to the glycoside bond chiefly affects the $C_{2'}$ chemical shift of ribose [2, 3]. This is due to polarization of the $C_{2'}$–H bond by the electrical field of the $C_{2'}$=O group of the pyrimidine base in the syn conformation. The changes in the chemical shifts in dimethyl sulfoxide (DMSO) in the spectra of cytidine (anti) and 6-methylcytidine (syn) are 4.9 and 1.2 ppm to the strong-field side for $C_{2'}$ and $C_{3'}$, respectively, and 3.4, 1.8, and 2.2 ppm to the weak-field side for $C_{1'}$, $C_{4'}$, and $C_{5'}$, respectively [3]. The effect of the solvent on the $C_{2'}$ chemical shift in the spectra of uridine and cytidine ($\Delta\delta C_{2'}$ = 1.6 ppm) is evidently also associated with a change in the conformation of the glycoside bond. According to the data obtained, the equilibrium in d_5-pyridine is shifted to the greatest extent to favor the anti conformation, while the greatest contribution of the syn conformation is observed in the more polar solvents d_6-DMSO and D_2O. The equilibrium in cytidine is shifted to favor the anti conformation to a greater extent than in uridine, in agreement with the data in [1].

The nature of the changes in the $C_{1'}$ chemical shift observed in the spectra of oligonucleotides as a consequence of conformational transitions was not ascertained in [4, 5]. However, the effect of the solvent on the $C_{1'}$ chemical shift is displayed most significantly.

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TABLE 1. Effect of Solvents on the ^{13}C Chemical Shifts of Uridine and Cytidine*

Nucleoside	Solvent	$C_{(2)}$	$C_{(4)}$	$C_{(5)}$	$C_{(6)}$	$C_{(1')}$	$C_{(2')}$	$C_{(3')}$	$C_{(4')}$	$C_{(5')}$
Uridine	d_6-DMSO	151,87	164,25	102,82	141,84	88,85	74,68	70,98	85,92	61,91
	d_7-DMF	151,90	164,36	102,56	141,80	89,38	75,21	71,33	86,22	62,25
	d_9-Trimethyl phosphate	151,90	163,98	102,50	141,50	89,70	75,08	71,31	86,19	62,21
	d_5-Pyridine	152,51	164,90	102,75	141,57	90,63	76,31	71,49	86,53	62,06
	d_4-Methanol	151,96	165,68	102,12	142,15	90,29	75,21	70,79	85,80	61,76
	D_2O	152,81	167,30	103,45	143,06	90,61	74,95	70,66	85,41	61,98
Cytidine	d_6-DMSO	156,73	166,68	95,27	142,67	90,29	75,17	70,61	85,27	61,80
	d_7-DMF	155,67	165,62	95,53	143,45	90,87	75,84	70,72	85,94	61,74
	d_5-Pyridine	157,45	167,55	94,39	142,71	92,72	76,77	70,88	86,11	61,72
	d_4-Methanol	157,77	167,00	95,16	142,53	91,81	75,56	70,04	85,18	61,26
	D_2O †	158,66	167,24	97,34	142,77	91,55	75,27	70,50	84,96	61,95

*Cyclohexane (δ 27.42 ppm) was used as the internal standard. The effect of the solvent on the chemical shift of the standard was disregarded, since it is insignificant.

†Dioxane (δ 67.8 ppm) was used as the internal standard in aqueous solutions.

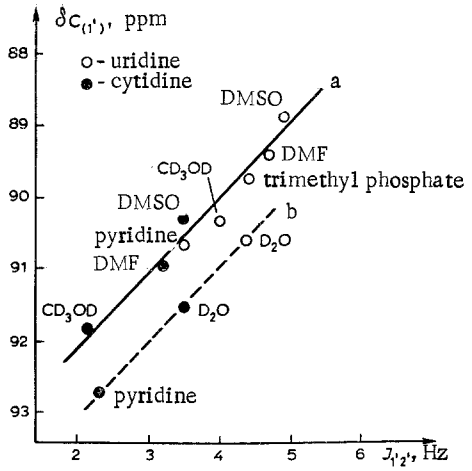

Fig. 1. Correlation of the C_1' chemical shifts with the $J_{1'2'}$ SSCC as a function of the solvent for pyrimidine nucleosides. The equation of the straight line was constructed by the method of least squares and is described by the formulas: a) $\delta C_1' = k \cdot J_{1'2'} + C$, b) $\delta C_1' = k \cdot J_{1'2'} + C + \Delta\delta C_1'$, where $k = -0.99$, $C = 94.0$ ppm, and $\Delta\delta C_1' = 1.0$ ppm.

We established a linear correlation between the C_1' chemical shift and the vicinal spin–spin coupling constants (SSCC) of the 1'-H and 2'-H protons (Fig. 1). It follows from data from a conformational analysis of nucleosides [10] that the orientation of the base changes from quasi-equatorial to quasi-axial as the conformation of the ribose ring changes from 2'-endo (S) to 3'-endo (N). On the basis of our observed correlation we assumed that the chief reason for the changes in the C_1' chemical shift is the nonidentical contributions of the α-effect [11] of the base as a consequence of a change in the conformational equilibrium of the ribose ring. One must also take into account the significant effect of the distribution of the electron density in the base on the C_1' chemical shift, as one can judge from the change in the chemical shifts in the spectrum of the base. The deviations in the case of aqueous solutions and the solution of cytidine in pyridine can be explained by this; the use of the correlation for the determination of the contribution of the effect of changes in the electron density in the base ($\Delta\delta C_1'$) to the C_1' chemical shift is possible in this case (Fig. 1). The effect of a change in the conformation of the glycoside bond on the C_1' chemical shift is evidently insignificant.

The data obtained confirm the previously established interrelationship of the conformational transitions in nucleosides [3] and indicate the effect of the nature of the solvent on the conformational equilibria in the investigated nucleosides. An increase in the shift of the conformational equilibria of the ribose ring of pyrimidine nucleosides of uridine and cytidine in favor of the 3'-endo (N) (anti) conformation in the following order of solvents is observed: d_6-DMSO > D_2O, d_7-DMF > d_9-trimethyl phosphate > d_4-methanol, d_5-pyridine.

EXPERIMENTAL

The ^{13}C NMR spectra were obtained with a Brucker WH-90 spectrometer at 22.63 MHz and 27 ± 1°C, the solvents (d_6-DMSO, d_5-pyridine, d_7-DMF, and d_9-trimethyl phosphate) were dried over 4-Å molecular sieves, and cyclohexane and dioxane (for the aqueous solutions) were used as the internal standards. The accuracy in the measurement of the chemical shifts was ±0.03 ppm. The concentrations of the solutions were no higher than 10%.

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HETEROCYCLIC NITRO COMPOUNDS.

27.* METHODS FOR THE SYNTHESIS OF 3-NITRO-5-R-1,2,4-TRIAZOLES

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A method for the preparation of 3-nitro-5-R-1,2,4-triazoles by reaction of 1-(3'-oxobutyl)-3,5-dinitro-1,2,4-triazole with nucleophilic reagents with various structures with replacement of the nitro group in the 5 position of the triazole ring during subsequent elimination of the oxobutyl fragment in an alkaline medium is examined. A number of previously undescribed NH acids, viz., 1,2,4-triazole derivatives, including 3-nitro-5-azolyl-1,2,4-triazoles, were obtained, and the ionization constants were determined for some of them.

One of the widely used methods for the synthesis of 3-nitro-5-R-1,2,4-triazoles is diazotization of 3-amino derivatives of 1,2,4-triazole and subsequent reaction of the diazo compounds with nitrite ion [2]. However, its application is not always possible in view of the limitations involved in varying the substituents in the 5 position of the starting amine. At the same time, it has been shown in the case of reactions of 1-methyl-3,5-dinitro-1,2,4-triazole with nucleophilic reagents [1, 3-5] that 3,5-dinitro-1,2,4-triazole derivatives can be used as starting substances for expanding the series of compounds of the triazole series.

*See [1] for Communication 26.

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