CONFORMATIONAL PROPERTIES OF THE 2'- AND 3'-HYDROXY GROUPS OF 5'-O-TRITYLURIDINE

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The results of a study of the temperature dependence of the SSCCs of the hydroxyl protons in the PMR spectra of 5'-O-trityluridine indicate the existence of an intramolecular bond between the 2-keto oxygen of the base and the 2'-hydroxyl in deuterochloroform. Under these conditions, stabilization of the 3'-endo (N) conformation of the ribose ring is observed. The existence of the influence of the temperature on the population of the 3'-hydroxyl rotamers shows the formation of a hydrogen bond between OH-3' and O-2'. In polar solvents free rotation of the hydroxy groups is observed.

In an investigation of the conformational properties of the hydroxy groups of the ribose rings of nucleosides [1-3], particular interest is presented by the possibility of the formation of an intramolecular bond between the 2'-hydroxyl and the base, stabilizing the conformation of the nucleic acids and their components [4]. However, there are only indirect proofs of the existence of this type of hydrogen bond [4]. Investigations of the conformational properties of the hydroxy groups of natural nucleosides by the PMR method have shown that in solutions of dimethyl sulfoxide (DMSO) and its mixtures with benzene there is practically free rotation of the 2'-hydroxyl [1, 2].

In the present communication we consider the conformational properties of the 2'- and 3'-hydroxy groups of 5'-O-trityluridine in dependence on the temperature in solvents of different polarities.

The conformation of the exocyclic groups at $C_2'-O_2'$ and $C_3'-O_3'$ bonds (see Fig. 1) can be established from the values of the spin–spin coupling constants (SSCCs) of the H–C–O–H' fragment [2]. The dependence of the SSCC $J_{HCOH'}$ on the dihedral angle φ between the planes of the H–C–O and the C–O–H' fragments is expressed by a modified Karplus equation [7]:

$$J_{HCOH'} = 10.4\cos^2\varphi - 1.5\cos\varphi + 0.2. \quad (1)$$

For a rapidly rotating hydroxy group, a mean SSCC over the three rotational states is observed:

$$J_{obs} = p_I J_{gauche} + p_{II} J_{gauche} + p_{III} J_{trans}, \quad (2)$$

$$1 = p_I + p_{II} + p_{III}, \quad (3)$$

where p_I, p_{II}, and p_{III} are the populations of the conformers I, II, and III, respectively, in molar fractions; and J_{gauche} and J_{trans} are the SSCCs of the gauche and trans conformers, respectively, Hz.

Making use of Eq. (1) the values $J_{gauche} = 2.1$ and $J_{trans} = 12.1$ Hz have been found previously. Thus, the population of the gauche and trans conformers p_{gauche} and p_{trans} can be determined from the equations

$$J_{obs} = 2.1\, p_{gauche} + 12.1\, p_{trans}, \quad (4)$$

$$1 = p_{gauche} + p_{trans}. \quad (5)$$

Let us consider the results on the SSCCs of the 2'- and 3'-hydroxylic protons of 5'-O-trityluridine given in Table 1. The constants in acetone-d_6 and DMSO-d_6 are equal and do not depend on the temperature in the temperature range considered. In addition to this, they are within the limits of the SSCCs for exocyclic hydroxy groups in solutions of natural nucleosides in DMSO-d_6 [1, 2]. Consequently, it may be concluded that in this case, also, there is free rotation of the 2'- and 3'-hydroxyls in view of the closeness of the observed values

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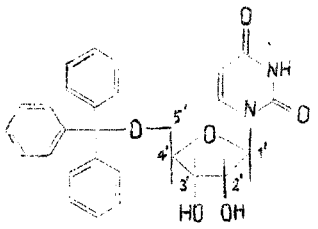

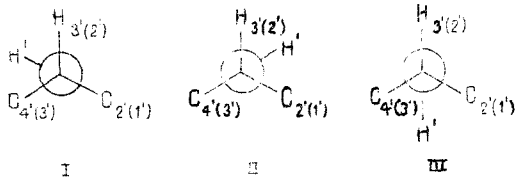

Fig. 1. Conformers at the $C_3'-O_3'$ ($C_2'-O_2'$) bond.

to the theoretical SSCC of a freely rotating hydroxyl (J = 5.4 Hz) found for the simplest aliphatic alcohols [7].

The coupling constants of the hydroxylic protons of 5'-O-trityluridine in deuterochloroform ($CDCl_3$) differ substantially from the values obtained in polar solvents. An investigation of the temperature dependence of the SSCCs of the 3'-hydroxyl has shown that the amount of trans rotamer increases sharply with a fall in the temperature (see Table 1). Such a change in the concentration of the rotamers apparently indicates the formation of an intramolecular hydrogen bond between the OH-3' and O-2', stabilizing the rotamers II and III (see Fig. 1), rotamer II proving to be energetically more favorable.

It is impossible to calculate the relative amounts of rotamers I and II by using Eqs. (4) and (5), but it may be assumed that the concentration of rotamer I will decrease with a lowering of the temperature faster than that of rotamer II in view of the additional stabilization of the latter by a hydrogen bond. As we shall see, raising the temperature leads to an equalization of the relative concentrations of the rotamers, probably because of the weakening of the H bonds.

The SSCC of the 2'-hydroxyl of 5'-O-trityluridine in $CDCl_3$ (J = 3.5 Hz) is considerably smaller than that observed in polar solvents (J = 4.7 Hz). Consequently, we must take into account the possibility of the formation of intramolecular hydrogen bonds between the 2-keto oxygen of the base and the 2'-hydroxyl, stabilizing rotamers II and III (see Fig. 1). If it is assumed that there is rotation of the 2'-hydroxyl, then, making use of Eqs. (4) and (5), it is possible to calculate the concentration of the trans rotamer III (p_{trans} = 14%). Further, it may be assumed that in view of the formation of identical H bonds, states II and III are equivalent, i.e., $p_{II} = p_{III}$. Then the population of the rotamer I (p_I = 72%) considerably predominates. This permits the conclusion that in actual fact the stabilization of rotamer I takes place through the formation of a hydrogen bond with the 2-keto oxygen of the base. However, where there is rotation, with a rise in the temperature the SSCC must increase because of the equalization of the relative concentrations of the rotamers resulting from a weakening of the hydrogen bonds. In addition, it can be shown that the population of the rotamers of the 2'-hydroxyl must, when a H bond with O-3' exists, depend on the distribution of the rotamers of the 3'-hydroxyl. However, this is not actually observed, since $J_{2'OH}$ does not depend on the temperature. This gives grounds for assuming that the SSCC value observed in $CDCl_3$ probably corresponds to a hindered state of the 2'-hydroxyl with a dihedral angle of the H—C—O—H' fragment $\Phi = 50°$, determined by using Eq. (1). Here the second value of $\Phi = 120°$ is unlikely, since it corresponds to the eclipsed state.

Considerable interest is presented by the influence of an intramolecular hydrogen bond between the 2-keto oxygen of the base and the 2'-hydroxyl on the conformational equilibrium of the ribose ring. With a lowering of the temperature the SSCC value of the vicinal protons H-1' and H-2' characterizing the conformational equilibrium of the ribose ring [8] increases considerably (see Table 1). This shows an increase in the population of the 3'-endo (N) conformation probably because of the rise in the energy of the hydrogen bond between the 2-keto oxygen and the 2'-hydroxyl, since in the absence of intramolecular hydrogen bonds in polar

TABLE 1. Influence of the Solvent and the Temperature on the Populations of the Gauche and Trans Rotamers with Respect to the $C_3'-O_3'$ Bond, Observed SSCCs of the 2'- and 3'-Hydroxylic Protons and of the Vicinal 1'–2' Coupling

Solvent	T, °C	$J_{3'-OH'}$, Hz	$P_{gauche'}$ %	$P_{trans'}$ %	$J_{1'-2'}$, Hz	$J_{2'-OH'}$, Hz
Deutero-chloroform	−15	8.2	39	61	0.3	3.5
	−10	8.2	39	61	0,6	3.5
	0	7.9	42	58	1.2	3.5
	10	7.3	48	52	1.5	3.5
	20	6.4	57	43	2.0	3.5
	30	6.0	61	39	2.4	3.5
	40	5.3	68	32	2,9	3.5
Acetone-d_6	−20	5,0	71	29	3.4	4.7
	30	*	*	*	3.4	4.7
DMSO-d_6	30	5.0	71	29	3.0	4.7

*The signal of the 3'-hydroxyl is masked by the H-2' and H-3' signals.

solvents, and also in solutions of the natural nucleosides in DMSO-d_6, no such relationship is observed.

Thus, it may be concluded that in the case of pyrimidine derivatives of the components of nucleic acids the formation of an intramolecular hydrogen bond between the 2-keto oxygen and the 2'-hydroxyl leads to a stabilization of the 3'-endo (N) conformation.

EXPERIMENTAL

The PMR spectra were taken in the pulsed regime followed by Fourier transformation on a Bruker WH-90 spectrometer with a working frequency of 90 MHz. The solvents deuterochloroform, acetone-d_6, and DMSO-d_6 were dried over 4 Å molecular sieves. The accuracy of measurement of the SSCCs was ±0.3 Hz and of the temperature ±1°C.

SUMMARY

The conformational properties of the 2'- and 3'-hydroxy groups of 5'-O-trityluridine as functions of the temperature in deuterochloroform, acetone-d_6, and DMSO-d_6 have been studied by PMR methods. On the basis of figures for the SSCCs for the hydroxylic protons, it has been concluded that an intramolecular hydrogen bond exists between the 2-keto oxygen of the base and the 2'-hydroxyl in deuterochloroform. The results of a study of the temperature dependence of the SSCCs indicate the formation of a hindered state of the 2'-hydroxy group with a dihedral angle of the H–C–O–H' fragment $\Phi = 50°$. It has been established that in the case of pyrimidine derivatives of components of nucleic acids the formation of an intramolecular hydrogen bond between the base and the 2'-hydroxyl stabilizes the 3'-endo (N) conformation of the ribose ring. The substantial influence of the temperature on the relative concentrations of the rotamers of the 3'-hydroxy group in $CDCl_3$ is possibly a consequence of the formation of a hydrogen bond between OH-3' and O-2'. In this case, the trans rotamer is energetically the most favorable. In polar solvents, free rotation of the hydroxy groups is observed.

LITERATURE CITED

1. D. E. Davies and S. S. Danyluk, Can. J. Chem., 48, 3112 (1970).
2. R. Deslauriers and I. C. P. Smith, Can. J. Chem., 51, 833 (1973).
3. D. B. Davies and A. Rabozenko, J. Chem. Soc., Perkin Trans., 2, No. 15, 1703 (1975).
4. N. K. Kochetkov, É. I. Budovskii, E. D. Sverdlov, M. F. Simukova, N. F. Turginskii, and V. N. Shibaev, The Organic Chemistry of the Nucleic Acids [in Russian], Moscow (1970), p. 141.
5. N. F. Myasoedov, C. V. Sidorov, and A. F. Usatyi, Khim. Prir. Soedin., 204 (1979).
6. M. P. Schweizer, A. D. Broom, P. O. P. Ts'o, and D. P. Hollis, J. Am. Chem. Soc., 90, 1042 (1968).

7. P. R. Fraser, M. Kaufman, P. Morand, and G. Covil, Can. J. Chem., 47, 403 (1969).
8. C. Altona and M. Sundaralingam, J. Am. Chem. Soc., 95, 2333 (1973).
9. K. N. Fang, N. S. Kondo, P. S. Miller, and P. O. P. T'so, J. Am. Chem. Soc., 93, 6647 (1971).

INVESTIGATION OF THE INFLUENCE OF INTRAMOLECULAR ELECTROSTATIC INTERACTIONS ON THE CONFORMATIONAL EQUILIBRIUM IN PYRIMIDINE NUCLEOSIDES BY THE PMR METHOD

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The influence of solvents on the PMR spectra of uridine and cytidine has been studied. Because of intramolecular electrostatic interactions (IEIs) between the 2-keto oxygen and the freely rotating 2-hydroxyl, the position of the conformational equilibrium in the pyrimidine nucleosides but not in purine and deoxy nucleosides, depends substantially on the dielectric constant of the solvent and the size of the partial negative charge on the 2-keto oxygen of the base. It has been shown that an increase in the IEI leads to an increase in the 3'-endo (N) population of the ribose ring and to an increased influence of the temperature on the state of the conformational equilibrium.

Attempts are usually made to ascribe various conformational effects of nucleic acids and their components, nucleotides and nucleosides, mainly to the direct interaction between the base and the ribose and, in particular, between the 2-keto oxygen and the 2'-hydroxyl of the ribose in pyrimidine derivatives [1, 2]. However, such an approach does not permit an explanation of, for example, the difference in the states of conformational equilibrium of the ribose ring between corresponding derivatives of uridine (U) and cytidine (C) [2].

We have studied the influence of intramolecular electrostatic interactions (IEIs) on the state of the conformational equilibrium in U and C. With this aim we have investigated the PMR spectrum of U and C in a number of solvents: (dimethyl sulfoxide)-d_6 (DMSO-d_6), dimethylformamide-d_7 (DMFA-d_7), methanol-d_4, pyridine-d_5, and water-d_2, since, according to the theory of reaction fields [3], the energy of an IEI depends on the dielectric constant of the medium ϵ.

The conformation of the ribose ring of a nucleoside can be described on the basis of the idea of pseudorotation [4], making use of the phase angle of pseudorotation P and the degree of pucker τ. In solutions, however, there is a dynamic equilibrium between conformers of the N and S types (Fig. 1), which include the classical 2'-endo (S) and 3'-endo (N) conformations [2]. When the condition that P and τ are constant is observed, the populations p_N and p_S of conformers of type N and S, respectively, can be calculated by using the "direct method" [2]:

$$p_S = 10\ J_{1'-2'}, \quad (1)$$

$$p_N + p_S = 1. \quad (2)$$

It is considered that this condition is satisfied if $J_{2'-3'}$ and $\Sigma = J_{1'-2'} + J_{3'-4'}$ are constant. Unfortunately, it was impossible to check the fulfillment of this condition, since the H-2' and H-3' signals partially overlap, which considerably complicates the analysis of the spectrum. However, it is known [5] that IEIs have little effect on the positions of the minima in space of the geometric parameters characterizing the conformations of molecules (in this case, on P and τ) but substantially change the absolute values of the energy. Consequently, we have assumed that τ and P do not depend on the solvent.

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