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Nucleoside conformers in low-temperature argon matrices: Fourier transform IR spectroscopy of isolated thymidine and deuterothymidine molecules and quantum-mechanical calculations

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ABSTRACT

The conformational equilibrium of thymidine and deuterothymidine molecules in low-temperature Ar matrices has been studied using low-temperature matrix-isolation Fourier IR spectroscopy and quantum-chemical calculations by the DFT/B3LYP and MP2 methods. It has been found that two anti-conformers ta2_0 and ta3_0 with different structures of the sugar ring, C2’-endo and C3’-endo, predominate in low-temperature matrices. In isolated state, each of these conformers has a few low-barrier satellites that can fully pass into more stable structures when a molecule enters the matrix. The main syn conformer ts2_0 is stabilized by an intramolecular hydrogen bond between the O5’H group of the sugar and the C2O group of the base (O5’H⋅⋅⋅O2), while C2’-endo is the predominant conformation of the deoxyribose ring. The considerably lower population of ts2_0 compared to the anti-conformers ta2_0, ta3_0 can be explained by the smaller population of satellite conformations. It has been shown that the absorption band of νN3D stretching vibration is split by the Fermi resonance.

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I. INTRODUCTION

Studies of the structure of constituent fragments of biopolymer molecules in isolated state are of fundamental interest for the contemporary science.1–7 For example, such structural elements of DNA and RNA as nucleosides are studied using the most up-to-date high-sensitive spectroscopy methods.6,7 A nucleoside consists of a heterocyclic DNA base bound to a five-membered furanose ring (ribose or deoxyribose) by a glycosidic bond.1 Even a minor modification of the base or furanose ring can significantly affect the conformational structure and biological functions of the nucleoside. Modified nucleosides are used to create new pharmaceuticals.8 The presence of a glycosidic bond predetermines the lower thermal stability of nucleosides in comparison with DNA bases. Laser evaporation is generally used to transfer such thermostable molecules to an isolated state.9,10 We have shown previously that the classical matrix isolation spectroscopy method9 and the thermodynamically equilibrium process of evaporation from a Knudsen cell can be used to study nucleosides in isolated state.

As a structural fragment, the thymidine nucleoside (Fig. 1) is a subject of numerous studies.10–13 The structure was mainly studied using polycrystalline samples, solutions, and quantum chemical computational methods.12,13 In the first experimental studies performed using the matrix isolation method,9,14 it was shown that anti- and syn- conformers of thymidine with various intramolecular hydrogen bonds were present in the matrices (O5’H⋅⋅⋅O2, O5’H⋅⋅⋅O3’, O3’H⋅⋅⋅O5’). However, no detailed analysis of the population of thymidine conformers detected in the matrix was carried out. Nucleosides are known to have a very complex conformational structure in isolated state.13 For example, quantum chemical calculations using the DFT/B3LYP/6-31G(d,p) and MP2/6-311++G(d,p)//DFT/B3LYP/6-31G(d,p) methods show that thymidine has 92 stable conformers.11 There are 22 conformers in the relative energy range of 0–1.5 kcal/mol11 and only some of them have clearly distinguishable characteristic bands. Specific features of matrix isolation spectroscopy allow even such
II. EXPERIMENTAL AND CALCULATION METHODS

The low-temperature Fourier IR spectra were measured using the experimental setup described previously.14,21,22 In this study, Fourier IR spectra of isolated thymidine (Thy) and O5’-O3’-, N3-deuterothyridine (Thy_d3) molecules in Ar matrices were recorded with an apodized resolution of 0.4 cm⁻¹ in the ranges of 450–2700 and 1400 – 4000 cm⁻¹. In addition, the IR Fourier spectra of thymine and 1-methylthymine molecules isolated in Ar matrices were recorded. The molecular fluxes of biomolecules and inert gases were measured using a low-temperature quartz microbalance.11,22 The evaporation system allowed us to obtain a molecular flow of Thy molecules with an intensity of 50–70 ng/(s·cm²) at temperatures of 415–435 K. The purity of the inert gas (Ar) was no worse than 99.99%.

Quantum chemical calculations were performed using the Gaussian16 programs24 and Firefly programs (version 8.0)25 that partially use the code of the GAMESS program (US).26 Part of the calculations was carried out at a personal workstation and on the grid cluster of B. Verkin Institute for Low Temperature Physics and Engineering of the National Academy of Sciences of Ukraine. The calculations of the vibrational spectra in a harmonic approximation and with consideration for the anharmonicity of oscillations were performed by a supercomputer cluster at the University of Arizona. The vibrational spectra were calculated using the 6-311++G(df,pd) basis with a dimension of 743 basis functions. The dimensions of correlation-corrected bases were 325, 551, and 1285 basis functions, respectively. The calculations were performed by density functional (DFT/B3LYP) and second-order Møller-Plesset perturbation theory methods (MP2). The standard capabilities of the GAMES program were used to estimate the relative free energies (ΔG) of conformers.

The torsion angles in the Thy molecule are shown in Fig. 1: C4’-C5’-O5’H (ζβ), C3’-C4’-C5’-O5’ (ζγ), O4’C1’N1C2 (ζζ), C4’-C3’-O3’H (εε). The designations of these angles and the method for calculating the pseudo rotation angle of the ribose ring (ζP) comply with the Saenger’s monograph. The conformations with anti-rotation of the pyrimidine ring around the glycosidic bond and with syn-rotation are denoted as tan_x and tsn_x, respectively. In these designations, n indicates a non-planar ribose conformation (2-C2’-endo or 3-C3’-endo) and x represents the numbers ordered by an increase in the relative energy of the conformers.

To estimate the intensity of absorption lines in experimental spectra, complex contours of spectral lines were approximated by Gaussian functions using the FITYK program.28 The SYNSPEC program was used to convert the calculated spectra to a superposition of Gaussian contours followed by comparison with the experimental spectra.

III. RESULTS AND DISCUSSION

Figure 2 shows the region of stretching vibrations of Thy molecules in an Ar matrix. The spectra of 2’-deoxyuridine (2_dU) and a complex system to be analyzed.15 When an impurity molecule is cooled in the matrix, some conformers can pass to lower-energy levels and the number of conformations decreases. Due to the high resolution of the matrix isolation method, it allows one to determine the absorption bands of OH and NH groups involved in the intramolecular hydrogen bonds that characterize the molecular structure of certain conformers. To determine the structure, the calculated and experimental vibrational spectra are compared. Using this scenario, we studied a number of pyrimidine and purine nucleosides (20 compared. Using this scenario, we studied a number of pyrimidine and purine nucleosides (20 compared. Using this scenario, we studied a number of pyrimidine and purine nucleosides (20 compared. Using this scenario, we studied a number of pyrimidine and purine nucleosides (20 compared. Using this scenario, we studied a number of pyrimidine and purine nucleosides (20 compared. Using this scenario, we studied a number of pyrimidine and purine nucleosides (20 compared. Using this scenario, we studied a number of pyrimidine and purine nucleosides (20 compared. Using this scenario, we studied a number of pyrimidine and purine nucleosides (20 compared. Using this scenario, we studied a number of pyrimidine and purine nucleosides (20 compared. Using this scenario, we studied a number of pyrimidine and purine nucleosides (20 compared. Using this scenario, we studied a number of pyrimidine and purine nucleosides (20 compared. Using this scenario, we studied a number of pyrimidine and purine nucleosides (20 compared.
1-methylthymine and thymine bases are provided for comparison. The spectrum of 1-methylthymine allows an unambiguous identification of the νN3H vibration. The spectrum of thymine is provided to control the thermal destruction. The thermal destruction of nucleosides can be detected by the appearance of narrow absorption bands of the corresponding base in the spectrum (after breakdown of the glycosidic bond). A comparison of the spectra of Thy and thymine shows (Fig. 2) that no thermal destruction was observed in our experiments with an evaporation temperature range of 415–435 K.

The conformational equilibrium of the gas phase can be preserved in a low-temperature matrix (or a supersonic beam) only provided that the barriers between the conformers are sufficiently high. Let us consider the possibility of a transition (interconversion) between the ts2_0 and ta2_0 conformers shown in Fig. 1. Figure 2 shows a good coincidence of the shapes and positions of the spectral bands of Thy and 2_dU. This suggests that the conformational compositions of these nucleosides in the matrix are similar. Using the potential energy surface map in the coordinates of the angles χ and β, it was previously shown that the transition barrier between the analogous 2-deoxyuridine conformers exceeds 4 kcal/mol. In this paper, this assessment was made by varying the χ angle in various directions. As shown in Fig. 3, the calculated barrier height between the ts2_0 and ta2_0 conformers exceeds 6 kcal/mol. Experiments show that annealing of matrix samples at a temperature range of 415–435 K does not significantly affect the intensity of the characteristic bands of thymidine. This agrees with the experimental data obtained by the matrix isolation method for small molecules that estimate the boundary value of the barrier as 2.5–3 kcal/mol for a temperature of 30 K. Hence, it can be concluded that, under the experimental conditions we used to create the sample, the interconversion between the thymidine syn- and anti-structures is completely absent.

However, the existence of low barriers is more likely within the syn and anti conformational families, since small fragments can rotate in them around angles ξβ, ξγ and ξε, and also the angle of pseudo rotation of the ribose ring (ξP) can change (Fig. 1). It was previously shown that low-barrier conformers of nucleosides are fixed in low-temperature matrices more poorly than conformers of small molecules. For example, the syn-conformer of 2'-deoxyuridine with C3'-endo structure of the sugar ring (the barrier of transition to C2'-endo is 1.3 kcal/mol) is not preserved upon freezing in Ar and Kr matrices even at 6 K. Moreover, transition of the O3'H hydroxy group of 2'-deoxyuridine molecules through a 1.2 kcal/mol barrier was observed upon deposition into a Kr matrix at 6 K. Approximate estimates of the interconversion time show that in order to fix low-barrier conformers, the cooling time should be comparable to the period of phonon oscillations of the matrix and low-frequency vibrational modes of molecules. The effect of the matrix on the energy relaxation in impurity molecules was already observed experimentally. It can be assumed that the energy relaxation ("cooling") rates of individual vibrational modes in complex molecules will differ. In fact, unlike 2'-deoxyuridine, a uridine syn-conformer with a C3'-endo sugar ring structure and a barrier height of 2.1 kcal/mol was fixed in an Ar matrix, and its interconversion was only observed upon annealing to 30 K. Therefore, the minimum barrier height that ensures the fixation of low-barrier nucleoside conformers in Ar and Kr matrices can be estimated as 2 kcal/mol.
conformational families can be fixed in matrices. At the same time, the barrier height between the syn-conformers ts2_0 and ts3_0 is smaller than 1.5 kcal/mol [Fig. 4(b)].

The experimental spectra contain no characteristic absorption bands of ts3_0 near 3520–3530 cm⁻¹ (Fig. 2). Therefore, we can assume that conformer ts3_0 passes into ts2_0 upon thymidine freezing in the matrix. This agrees with the results obtained for similar syn-structures of 2’-deoxyuridine in Ar and Kr matrices.¹⁴

The conformational pattern within each ta2 and ta3 family is determined by the rotations of the hydroxymethyl group (ζγ) and hydroxy groups O5’H (ζβ) and O3’H (ζε). Figure 5(a) shows a fragment of the potential energy surface for the ta2 family that has the shape of three ravines stretched along the abscissa axis (ζβ). The ravines are separated by high barriers. Inside each ravine there is only one minimum that can be fixed in the matrix [Fig. 5(b)]. The ta2_0 conformation is the global minimum of this family and has two low-barrier satellites (< 0.5 kcal/mol) that will pass to ta2_0 upon cooling in the matrix. The conformations of ta2_1, ta2_2 (Fig. 5) and their satellites are significantly lower in energy than ta2_0 (> 2 kcal/mol) and have no well-defined characteristic bands.

The potential energy surface of the anti-conformer family with C3’-endo structure of the deoxyribose ring is shown in Fig. 6(a). The ta3_0 conformation is the global minimum of this family. Unlike the ta2_0 conformer, the low-barrier satellites of ta3_0 are much closer in energy to the global minimum [Fig. 6(b)]. Though the energy of the ta3_0 conformation is lower, the ta3_1 and ta3_2 structures are interesting for further consideration. Like in the case of 2’-deoxyuridine,¹⁵ these conformations are characterized by intramolecular hydrogen bonds O5’H···O3’H and O3’H···O5’H. The weak absorption band near 3600 cm⁻¹ in the spectrum can correspond to these bonds (Fig. 2).

There are no clearly defined extended ravines on the surface maps of the potential energy of thymidine syn-conformers plotted as functions of β and γ angles (Fig. 7). The ts2_0 conformation is the global minimum of the family of syn-conformers with the C2’-endo structure of the deoxyribose ring [Fig. 7(a)]. Obviously, it has no satellites caused by rotation of the O3’H hydroxy group (ζβ). The low-barrier satellite of this conformation is ts3_0, the global minimum of the syn-conformer family with the C3’-endo structure of the deoxyribose ring [Fig. 7(b)]. The ts2_1 structure on the surface map of this family is of interest. A specific feature of this structure is that the hydrogen atom of the O3’H hydroxy group can interact with O4’, perhaps through a very weak intramolecular hydrogen bond. As a result, the absorption band of the stretching vibrations of νO5’H can shift to the 3620–3640 cm⁻¹ region. However, the stability of this structure during deposition into a matrix is not guaranteed. The ts3_1 and ts3_2 structures [Fig. 7(b)] are syn-analogues of minor anti-conformations ta3_1 and ta3_2 with O5’H···O3’ and O3’H···O5’ intramolecular hydrogen bonds, respectively. However, they are inferior in energy to these conformations, therefore they are not discussed below in detail.

The ta2_0, ta3_0, and ts2_0 conformations possess not only the low-barrier structural satellites discussed above (Figs. 4–7). Additional conformational structures can be formed by rotation of the O3’H hydroxy group that is reflected by angle ζε. The profiles of barriers of conformational transitions formed by this rotation are shown in Fig. 8. One can see that each basic conformation (ta2_0, ta3_0, ts2_0) has one low-barrier satellite which is certainly unstable upon deposition into a matrix. Moreover, each basic conformation has one structure with a 1.55–1.6 kcal/mol barrier (Fig. 8). Given this barrier height, it is difficult to accurately predict the behavior of a conformer upon deposition into a matrix. These satellite structures have no well-defined characteristic bands, therefore more detailed calculations were not carried out for these structures.

For the ta2_0 [Fig. 8(b)] and ta3_0 [Fig. 8(c)] conformations, the angle ζε is close to 180°. For ts2_0, two almost identical minima can be seen [Fig. 8(a)]. The ts2_0 structure with the ζε angle of 300° [Fig. 8(a)] was chosen based on additional calculations by the MP2/aug-cc-pVDZ method.

Based on the analysis made, six basic conformations of thymidine were selected for more accurate calculations of relative energies (using the MP2 method) and vibrational spectra (Table I). Although the ts3_0 conformer passes into the ts2_0 structure, these calculations were performed for it as well in order to determine more accurately the population of ts2_0 in low-temperature conditions.
FIG. 5. The surface map of the potential energy of thymidine anti-conformers with the C2'-endo structure of the deoxyribose ring. The calculation of the surface (a) and the profile of ravines (b) was performed using the DFT/B3LYP/cc-pVDZ method. The surface isolines are marked in kcal/mol.

FIG. 6. The surface map of the potential energy of thymidine anti-conformers with the C3'-endo structure of the deoxyribose ring. The calculation of the surface (a) and the profile of ravines (b) was performed using the DFT/B3LYP/cc-pVDZ method. The surface isolines are marked in kcal/mol.
matrices. With the same purpose, DFT calculations were performed for a number of structural satellites of the main conformers. The results of energy calculations presented in Table I show that the ts2_0 conformer is the most stable structure, regardless of the calculation method. However, taking into account the corrections for the Gibbs free energy at the evaporation temperature used in the experiment (420 K), it follows that the free energy global minimum corresponds to the ta3_0 conformer (Table I). To calculate the populations of conformers in the gas phase, the following formula was used:

$$N_j(T) = \frac{k_j e^{-\Delta G_j(T)/RT}}{\sum_{i=0}^{n} k_i e^{-\Delta G_i(T)/RT}} \times 100\%,$$

(1)

where $k_j$ is the coefficient of degeneration of conformation $j$.

The populations were calculated taking 7 low-barrier satellites (Figs. 5–8) for each anti-conformation (ta2_0 and ta3_0) and 4 satellites for the syn-conformation (ts2_0) into account. In order to take the population of syn-analogs of ta3_1 and ta3_2 conformations into account (Figs. 6 and 7) without complicating the analysis, it was assumed that these conformations are twice degenerate. As a result, calculations by Eq. (1) predict a noticeable dominance of anti-conformations in the matrix samples (Table I). The calculated values of $\Delta G$ and, accordingly, the occupancies can be checked using the equation that partially uses experimental data:

$$\Delta G_{AB}(T) = -RT \ln K_{AB} = -RT \ln \left( \frac{I_A \cdot I_B}{I_{A,C} \cdot I_{B,C}} \right),$$

(2)

where $I_{A,E}$, $I_{B,E}$ are the experimental intensities of characteristic bands of conformers $A$ and $B$; $I_{A,C}$ and $I_{B,C}$ are the calculated intensities presented in Table II.
TABLE I. Structural parameters (characteristic angles) and relative energies (ΔE, ΔG in kcal/mol) of the basic thymidine conformers.

<table>
<thead>
<tr>
<th>Torsion angles</th>
<th>Conformers</th>
<th>ta2_0</th>
<th>ta3_0</th>
<th>ta3_1</th>
<th>ta3_2</th>
<th>ts3_0</th>
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<td>Energies and calculation method</td>
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*Averaged ΔE values calculated by the MP2/aug-cc-pvdz and MP2/aug-cc-pvtz methods.

TABLE II. Experimental frequencies (cm⁻¹) and intensities of the absorption bands in the range of stretching vibrations νOH, νNH, νC=O, νC5C6 of Thy (Thy_d3) molecules in Ar matrices, as well as the vibration frequencies and intensities of the main Thy (Thy_d3) conformers calculated by the DFT/B3LYP/6-311++G(df,pd) method.

<table>
<thead>
<tr>
<th>Vibration</th>
<th>Conformer</th>
<th>ta2_0</th>
<th>ta3_0</th>
<th>ta3_1</th>
<th>ta3_2</th>
<th>ts2_0</th>
<th>Ar matrix</th>
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<tbody>
<tr>
<td></td>
<td>v, cm⁻¹</td>
<td>I, a.u.</td>
<td>v, cm⁻¹</td>
<td>I, a.u.</td>
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<td>3643</td>
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<tr>
<td>vO3H– hydrogen bond with O5'</td>
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<tr>
<td>vO3H– hydrogen bond with O2'</td>
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<td>vO3H– hydrogen bond with N3</td>
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<td>vN3H</td>
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<tr>
<td>v(C5 = C6)</td>
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</tr>
</tbody>
</table>
| Note: I, is the calculated intensities, km/mol; I, is the integral experimental intensities. The calculated vibration frequencies νOH, νNH were multiplied by a correction factor of 0.95168 (0.96434 for νOD, νND Thy_d3); the νC=O, νC5C6 vibration frequencies were multiplied by 0.978. The numbers in parenthesis is the Thy_d3 frequencies.
| aAnharmonic frequencies based on the anharmonic problem solution using the vibrational second-order perturbation theory (VPT2).
The absorption band of the experimental spectrum with a maximum at 3664 cm\(^{-1}\) represents the superposition of the absorption bands of the most populated anti-conformers ta2_0, ta3_0, and ta3_1 (Table II). This allows one to adjust the total population of the syn- and anti-subsets of conformers using Eq. (2). The averaged intensity of the O5'H vibration band equal to 50 km/mol was chosen to perform the correction.

As a result, the population values obtained matched rather well the data obtained solely by calculations (Table I).

Based on the data of the population of conformers in the matrix, it can be seen that the majority of bands in the experimental spectrum belongs to the ta3_0, ta2_0, and ts2_0 conformations. Therefore, Table II presents the calculated characteristics of the absorption bands of the stretching vibrations of the OH, NH, and CO groups in these conformations. In the range of vOH, vNH stretching vibrations, a DFT/B3LYP/6-311++G(df,pd) calculation in harmonic approximation shows a good agreement between the calculated and experimental frequencies if the correction factor ("scaling factor") of 0.95168 is used (Table II). The calculated spectrum of thymidine is also in good agreement with the experimental spectrum [Fig. 9(a)]. The calculated spectrum was visualized for superposing the absorption bands of oscillations of the 5 main conformers with consideration for their population (Table I). To visualize the calculated absorption band, a half-width of the Gauss formers with consideration for their population (Table I). To visualize the calculated absorption band, a half-width of the Gauss formers with consideration for their population (Table I).

In the spectrum of Thy_d3, the absorption bands of the vOD and vND vibrations shift to 2700–2500 cm\(^{-1}\) and their intensities decrease markedly [Fig. 9(b)]. The results of spectral calculations by the DFT/B3LYP/6-311++G(df,pd) method in a harmonic approximation and with correction by a factor of 0.96434 well agree with the experimental spectrum [Fig. 9(b) and Table II]. This is observed for all the spectral bands in this range, except for the vN3D vibration band [Fig. 9(b)]. The absorption band of this vibration overlaps with the vO5'D band of the ts2_0 conformer [Fig. 9(b)] and is split into two bands with frequencies of 2552 and 2533 cm\(^{-1}\) [Fig. 9(b) and Table II]. The frequencies of these two bands are shifted in different directions from the calculated frequency of 2543 cm\(^{-1}\) of vN3D vibrations [Fig. 9(b) and Table II]. This splitting may result from the Fermi resonance. It is facilitated by the fact that, in comparison with vN3H, the vN3D vibration frequency is shifted to the 2500–2800 cm\(^{-1}\) range where a few variants of combinational bands are possible.\(^{23}\) Previously, we have already detected a splitting of the vN3D vibration band for deuterated 5I-uracil caused by the Fermi resonance.\(^{23}\) Calculations show that the frequencies of vCO stretching vibrations insignificantly differ from each other (Table I). Therefore, the vC0 vC2O and vC4O vCO in the synthesized calculated spectrum are well separated [Fig. 10(a)]. However, nearly a single broad absorption band is observed in the experimental spectrum [Fig. 10(a)]. This broadening of the absorption bands of vCO vibrations can also be caused by the Fermi resonance that often manifests itself in this region of the spectrum of pyrimidine bases.\(^{21}\) In particular, the Fermi resonance produces 5 additional absorption bands in the spectrum of 1-methylthymine near the absorption bands of vCO fundamental vibrations [Fig. 10(a)]. The results of anharmonic calculations by the VPT2 method also indicate the possibility of the Fermi resonance in this spectral region. According to the calculations for vC2O and vC4O vibrations of ta2_0 and ta3_0 conformers, the Fermi resonance with several Raman modes is possible. These modes are formed by bending vibrations of the pyrimidine ring whose frequencies lie in the ranges of 1100–1295 and 480–650 cm\(^{-1}\).
Deuteration can significantly change the resonance conditions, both due to the effect of certain vibrations on anharmonicity and due to a significant shift in the frequencies of combination vibration bands. The experimental spectrum of Thy_d3 manifests a distinct separation of absorption bands of the vC2O and vC4O vibrations and hence a good agreement between the experimental and calculated spectra [Fig. 10(b)].

IV. CONCLUSION

Using IR Fourier spectroscopy of molecules in inert matrices and quantum-mechanical calculations, it has been shown that the equilibrium in the gas phase between the main anti- and syn-conformers of thymidine is preserved upon freezing in inert Ar matrices. The overall populations of anti- and syn-conformers in inert Ar matrices at temperatures of 6–11 K are ca. 80 and 20%, respectively.

Two anti-conformers of thymidine differing in the structures of the deoxyribose ring: C2'-endo and C3'-endo, dominate. These conformers have several structural satellites with low barriers that can completely pass into the main structures when thymidine molecules enter the matrix. Owing to the interconversion of satellite structures, the population of anti-conformers with a C3'-endo deoxyribose ring in the matrix can significantly exceed the population of similar C2'-endo structures.

The C2'-endo conformation of the deoxyribose ring is predominant for syn-conformers. The fact that the population of anti-conformers in the ts2_0 syn-conformer is lower can be explained by the smaller number of low-barrier satellite conformations.

The spectra of deuterothyminidine show an overlapping of the vN3D vibration bands and the stretching vibration of the O5'D group of deoxyribose that is linked by an intramolecular hydrogen bond to the C2O group of the pyrimidine base ring. This is facilitated by the splitting of the vN3D absorption band (2543 cm⁻¹) due to the Fermi resonance with the Raman modes of the pyrimidine ring.

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