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Low temperature Fourier transform infrared spectra and hydrogen bonding in polycrystalline uracil and thymine

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Abstract

The FTIR spectra of pure NH and isotopically diluted (NH/ND and ND/NH) polycrystalline uracil and thymine were measured in the range 4000–400 cm^{-1} at temperatures from 300 to 10 K. For the first time, the essentially narrow bands corresponding to the uncoupled stretching (ν_1) and out of plane bending (ν_4) NH proton modes of uracil and thymine were observed in the solid phase. It was found that in the ν_4 region the spectra reveal more details on the H-bond interactions present in both solids than in the ν_1 range. The frequencies of the various bands observed in both spectral regions were used for estimation of the H-bond energy, using empirical correlations between this property and both the red shift of ν_1 and the blue shift of ν_4 that occur upon crystallization due to the establishment of the H-bonds. The results are compared with known thermodynamic, structural and theoretical data. The IR data also suggest that the H-bond networks of both crystals contain, besides the two NH$\cdots$O=C bonds revealed by X-ray experiments, additional types of H-bonds, which do not show long range periodicity and, thus, cannot be detected by the conventional structural methods. The assignment of some other bands in the spectra of both substances was also reviewed.
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1. Introduction

This paper is part of a series where an approach based on the concerted use of low temperature FTIR spectroscopy and isotopic dilution techniques has been applied to the study of hydrogen bonding in crystalline networks of biological interest. The general approach used was formulated recently [1,2] and the previous results unequivocally demonstrate the existence of non-negligible H-bonding disorder in crystalline nucleosides [1], carbohydrates [3,4], L-alanine [5] and cytosine [6,7], which cannot be probed using standard structural methods (e.g., X-ray diffraction).

The main advantages of the methodology here used result essentially from the fact that the broadening generally observed in the bands originated in both the stretching (ν_1) and out of plane bending (ν_4) proton modes, which result from the interaction of these modes with thermally excited low frequency intermolecular librations, is suppressed at $T < 30$ K, leading to a considerable increase in the spectral reso-

lution. In addition, isotopic dilution provides a way to decouple individual proton modes, enabling the direct observation of bands originated in a particular proton vibration, which under normal conditions is coupled to vibrational modes of symmetrically equivalent protons [1]. As a result, under the conditions described the typical bandwidths of ν_1 and ν_4 bands decrease from hundreds to tens of wavenumbers, new details in the spectra become visible and the parameters of these bands can be measured with high accuracy.

In the present paper, the IR spectra of uracil and thymine were examined using the experimental conditions described above briefly. Particular emphasis is given to the analysis of the stretching and out of plane bending NH proton mode bands.

The atom numbering schemes used in this paper for the two molecules under analysis are shown on Fig. 1. Despite both uracil and thymine are constituents of nucleic acids and have attracted considerable interest in the past, the number of studies dealing with their hydrogen bonding structures in the crystalline phase is relatively scarce [8–11]. From X-ray diffraction experiments, it is known that the differences between hydrogen bond contacts in

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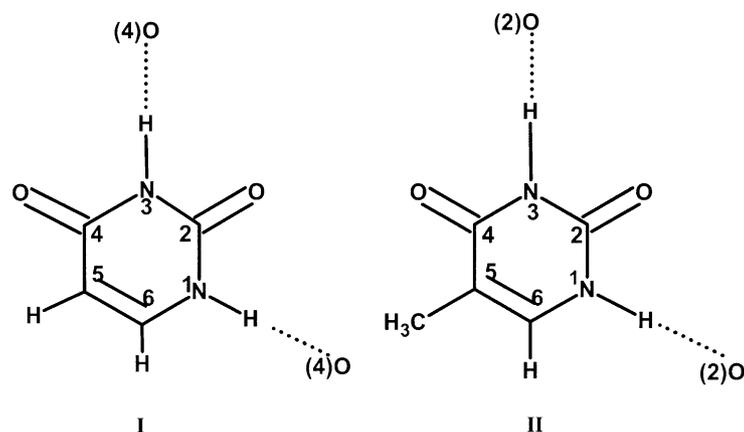*E-mail address:* markroz@chem.ch.huji.ac.il (M. Rozenberg).

Fig. 1. Chemical structure and atom numbering of uracil (I) and thymine (II).

the crystals of the two compounds are relatively small. The two compounds were found to exist, in crystals, exclusively in their diimino-diketo tautomer [8–11]. Uracil forms centrosymmetric dimers through N3–H $\cdots$ O=C4$'$ linkages (with N3 $\cdots$ O distances of 0.2865 nm), which are connected by additional bifurcated H-bonds involving the N1–H and O=C4 groups and identical partners from neighbor molecules (with N1 $\cdots$ O distances equal to 0.2864 nm) [9,10]. The O=C2 carbonyl group is involved in two weaker C–H$'$ $\cdots$ O=C2 interactions (the symbol ($'$) was here used to distinguish different molecules). For thymine, similar cyclic dimers with N3–H$'$ $\cdots$ O=C2$'$ and N1–H $\cdots$ O=C2 hydrogen bond contacts of 0.2810 and 0.2836 nm, respectively, were found to constitute the main structural unit of the crystal [10,11]. The most interesting features to stress here are (i) the fact that in the X-ray structures of both crystals each one of the N1–H and N3–H groups participates in a single type of H-bond, with a well defined characteristic distance, (ii) the observation that the dimers in uracil are made by H-bonds connecting N3 of one molecule to the O=C4 group of the other, while in thymine dimers the acceptor carbonyl group is O=C2 in both molecules but the donor atom is N1 in one molecule and N3 in the other (in this case, the dimers are not centrosymmetric).

Uracil and thymine were also studied previously by FTIR and Raman spectroscopy methods. Both compounds were studied as free molecules in the gaseous phase [12] and inert gas matrices [13–20]. They were also studied in the crystalline phase, both at room and low temperatures [15,21–23]. The assignment of the observed bands has been, in some cases, supported by theoretical predictions of the vibrational spectra for the isolated molecules [24,25] or for the crystal state [21,22,26]. In this latter case, the vibrational frequencies calculated for single molecules were afterwards empirically adjusted in order to fit the experimental data.

One important conclusion from previous spectroscopic studies on uracil and thymine refers to hydrogen bonding and can be synthesized using words from [18]: "...*the intermolecular forces in the solid have a profound effect on the appearance of the IR spectrum, such that correspondence between the bands observed in the solid and those observed here for the isolated molecules is not obvious*". This conclusion stressed the interest of using IR spectroscopy together with specifically designed sampling techniques, as isotopic dilution, to further elucidate the details of the hydrogen bonding networks in the crystals of the two compounds.

As mentioned before, in the present study, to overcome the well-known difficulties to estimate the peak positions of the usually very broad ν_1 and ν_4 bands and assign their different components, we took advantage of using isotopically doped crystals and low temperatures. The spectral changes were followed as a function of temperature, within the range 10–300 K.

2. Experimental

The IR spectra of polycrystalline pure NH- (Sigma), ≈5% ND- and 80–85% ND-uracil (or thymine) in a KBr (1:200) disc, attached to the cold finger of an APD Cryogenics closed-cycle helium refrigeration system with a DE-202A expander, were recorded with a Mattson Infinity 60AR series FTIR spectrometer, with spectral resolution of 1 cm^{-1}. The temperature (10–300 K) was measured directly at the sample holder by a silicon diode temperature sensor connected to a Lake Shore Cryogenics temperature controller (model DRC-91CA). The sample temperature during registration of spectra was kept stable within 0.2 K. The temperature-induced spectral changes observed for all substances were found to be reversible and highly reproducible. Deuterated samples were obtained from commercial uracil (thymine) by repetitive exchange with D$_2$O (Aldrich) in recirculating cyclohexane at 81 °C, as described in [27].

3. Results and discussion

In this study we are particularly interested to look at the temperature and isotopic shift effects on the NH proton modes. Hence, experimental conditions were chosen so that
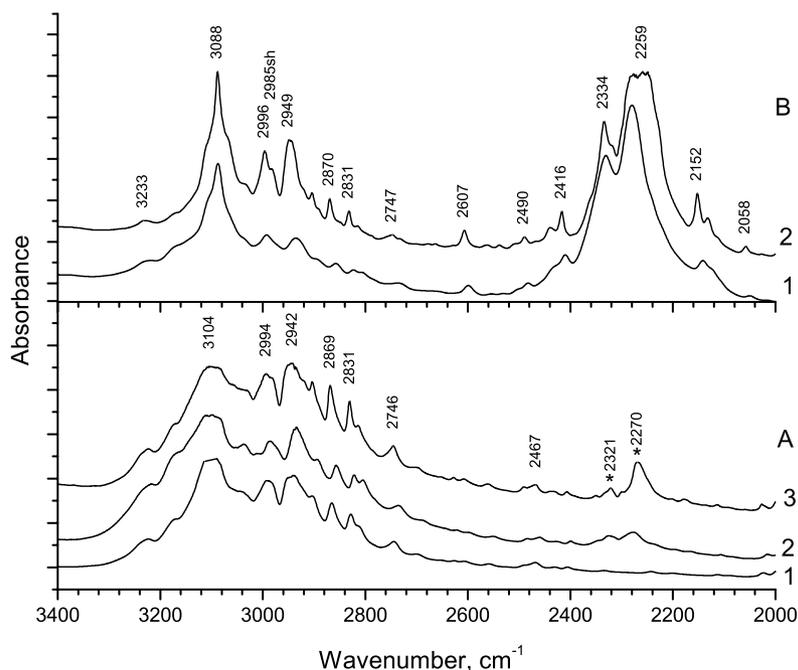
Fig. 2. FTIR spectra of uracil in the NH and CH stretching spectral region: (A) 1–100% NH at 300 K; 2–95% NH at 300 K; 3–95% NH at 10 K; (∗) bands of ND doped groups; and (B) 1–15% NH at 300 K; 2–15% NH at 10 K.

they were optimized to examine the usually very weak bands originated in the isotopically doped molecules appearing in the 3400–2000 cm^{-1} spectral range and below 1550 cm^{-1}. On the contrary, the spectral range 1700–1550 cm^{-1}, where very intense bands of uracil and thymine are observed (mainly νC=O and νC=C bands), has been studied in a multitude of previous works and will not be discussed in detail here.

3.1. Spectral assignments

3.1.1. 3400–2000 cm^{-1} spectral range: the NH and CH stretching vibrations

3.1.1.1. Uracil. The spectra of polycrystalline uracil samples in the 3400–2000 cm^{-1} region are shown in Fig. 2. Fig. 2A shows the spectra of the pure NH compound (trace 1) and of the ca. 5% ND doped crystal (traces 2 and 3, obtained at 300 and 10 K, respectively); Fig. 2B presents the spectra of the ca. 85% ND containing crystal (traces 1 and 2 correspond to $T = 300$ and 10 K, respectively).

From Fig. 2A (traces 2 and 3), it can be concluded that the very broad and complex band appearing in the 3400–2500 cm^{-1} region, whose intensity predominantly originates in the νNH stretching modes of samples with an NH concentration close to 100%, does not change significantly upon cooling. The main observed effect of lowering the temperature is the usual narrowing effect that leads to a slightly better resolved band structure. The same behavior is essentially observed for the ND bands of the 85% ND containing crystal of uracil shown in Fig. 2B, though

in this case the main peak (observed at 2270 cm^{-1} in the spectrum at room temperature) shows a noticeable red shift upon cooling. On the other hand, the bands originated in the isotopically doped molecules (both in the 5% ND and 15% NH doped crystals), which are, as expected, relatively narrow, show small but remarkable temperature changes. These changes are particularly evident in the case of the bands due to the ND containing molecules in the 5% ND doped crystal (see Fig. 2A, traces 2 and 3), which appear in a clear spectral region.

The strong differences between the NH or ND stretching band profiles originated in the dominant isotopic species (compare the 3400–2500 cm^{-1} NH stretching region in Fig. 2A with the 2400–2000 cm^{-1} ND stretching region in Fig. 2B) clearly indicate that the structures exhibited by these bands result mainly from resonant interactions. Resonance interactions involving overtones of the NH or ND bending modes are also observed in the case of bands due to the minor species in the doped crystals. The temperature dependence of the relevant spectral regions is shown with more detail in Fig. 3.

In the case where the NH containing molecules are the minor species present in the crystal (Fig. 3A), the bands observed at 2993 and 2936 cm^{-1} at 300 K experience blue-shifts upon cooling. These bands can be assigned to the overtones of the NH in-plane bending modes, which are strengthened by resonance interactions with the ν_1 (N3H) stretching mode and whose fundamentals are observed at 1516 and 1463 cm^{-1} (see Fig. 4). The feature with absolute maximum at 3088 cm^{-1} has at least three components: the narrow component at 3088 cm^{-1}, that does not change its
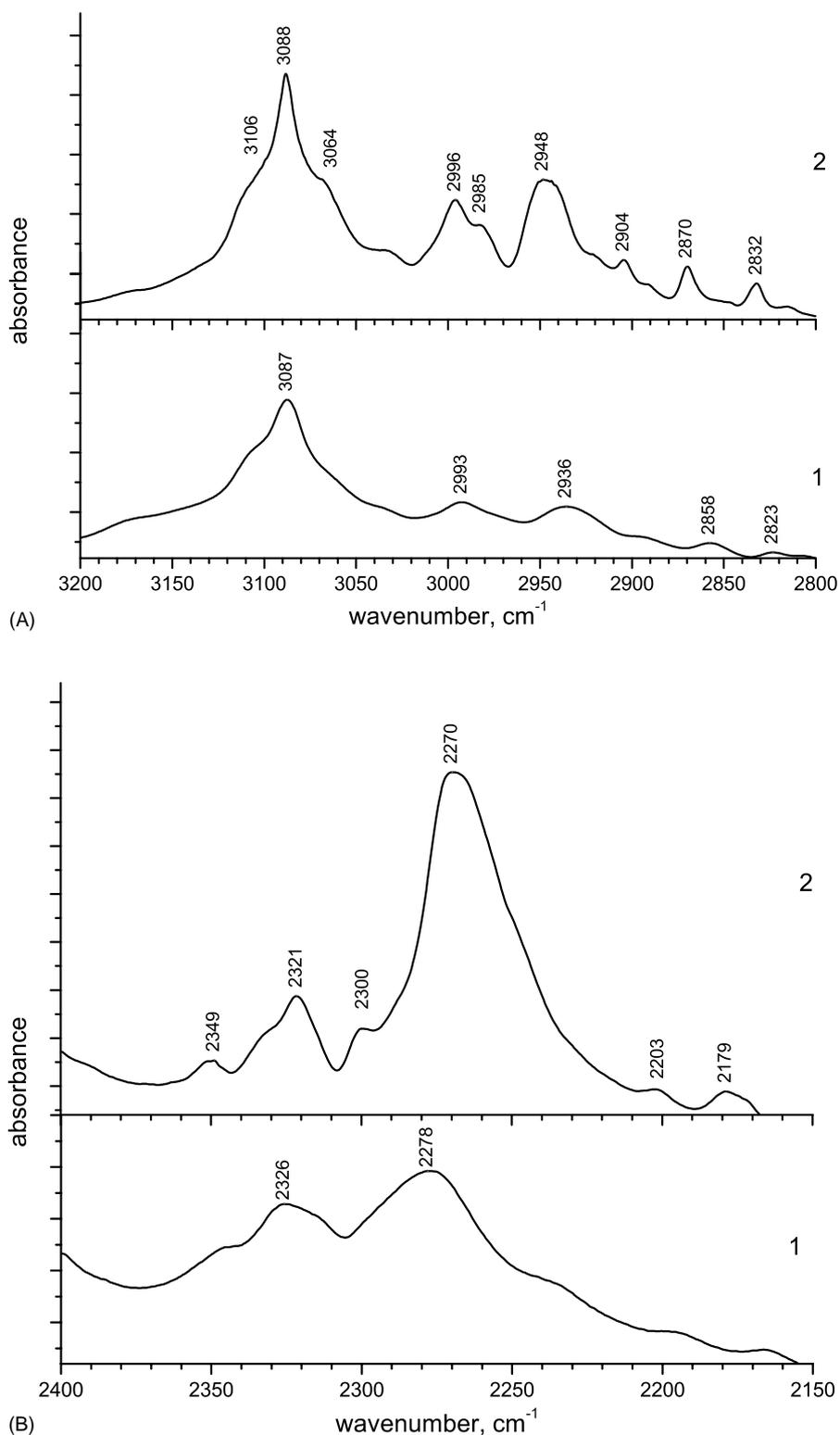
Fig. 3. Expansion of the NH and CH stretching spectral region: (A) ν_1 NH bands in the spectrum of mostly ND-uracil; 1—spectrum obtained at 300 K; 2—spectrum obtained at 10 K; and (B) ν_1 ND bands in the spectrum of mostly NH-uracil; 1—spectrum obtained at 300 K; 2—spectrum obtained at 10 K.

frequency and intensity upon cooling and shows a slight decrease in its bandwidth, corresponds to the CH antisymmetric stretching mode with controversial assignment to C6H [20] or to C5H [24]; the highest frequency compo-

nent, at 3106 cm^{-1}, which does not change in position and bandwidth but increases its intensity by ca. 60% on cooling, is ascribable to the ν_1 (N1H) stretching mode; finally, the lowest frequency component, at 3064 cm^{-1}, red shifts by

$4 \, cm^{-1}$, reduces its bandwidth (by ca. 10%) and intensifies (approximately two times), corresponding to an additional component of the whole set of bands resulting from resonant interactions involving the ν_1 (N3H) mode (as mentioned above, other bands resulting from this interaction are observed at 2993 and $2936 \, cm^{-1}$). Since the inherent overtone intensity involved in the resonant interaction with ν_1 (N3H) should be small, all three components shall be essentially described as ν_1 (N3H) bands; the frequency of the gravity center for this set of bands is then ca. $3000 \, cm^{-1}$. Note that the assignments here proposed indicate a larger relative IR intensity for the ν_1 (N3H) mode than for ν_1 (N1H). Hence, both relative frequencies and IR intensities of ν_1 (N3H) and ν_1 (N1H) indicate that the first group is involved in stronger H-bond interactions in crystalline uracil (it is well known that the intensity of the proton stretching vibrations of the groups involved in hydrogen bonding increases with the strength of the interaction [28]). The larger red shift observed for the ν_1 (N3H) mode relatively to its frequency for the matrix isolated uracil molecule when compared with the shift observed for ν_1 (N1H): 423 versus $364 \, cm^{-1}$ (taking as reference results obtained in a N_2 matrix [15]) or 433 versus 376 (using data obtained for the molecule in an Ar matrix [20]), is also consistent with a stronger H-bonding in N3H than in N1H.

The results are clearly more difficult to interpret in the case where the ND containing molecules are the minor species present in the crystal (Fig. 3B). Indeed, in this case the assignment of the observed bands to the ν_1 (N3D) and ν_1 (N1D) modes could not be unequivocally undertaken. The observed band profile consists essentially of two components, each one formed by several overlapping bands: the first component stays between 2370 and $2300 \, cm^{-1}$, and the second component comprehends the region between 2300 and ca. $2200 \, cm^{-1}$. At cooling, the intensity ratio of the two components changes from 1:2, at 300 K, to ca. 1:5 at 10 K. A possible interpretation for, at least, part of this change in the relative intensities, suggests that ν_1 (N1D) and ν_1 (N3D) contribute mainly to the higher frequency and lower frequency set of bands in this region, respectively; the strength of the ν_1 (N3D) H-bond increases in a greater extension with the decrease in temperature than that involving ν_1 (N1D); accordingly, the low frequency/high frequency region intensity ratio also decreases. Note that this tentative assignment of the ν_1 (N1D) and ν_1 (N3D) modes to the high and low frequency set of components in this spectral region leads to isotopic shifts of ca. 1.33 for both vibrations, which are identical to those previously observed for alanine and cytosine vibrations [5,6]. Another phenomenon that may also contribute to the observed change in the relative intensity of the two main components found in this spectral region involves resonance interactions of ν_1 (N3D) with the overtone of the ND in-plane bending mode (whose fundamental is observed at $1147 \, cm^{-1}$—see Fig. 4, trace 3). This resonance is expected to be sharper at 300 K (leading to a larger distribution of intensity between the two components), while, on cooling, the overtone moves upwards and the ν_1 (N3D) band moves downwards in frequency, weakening the resonance
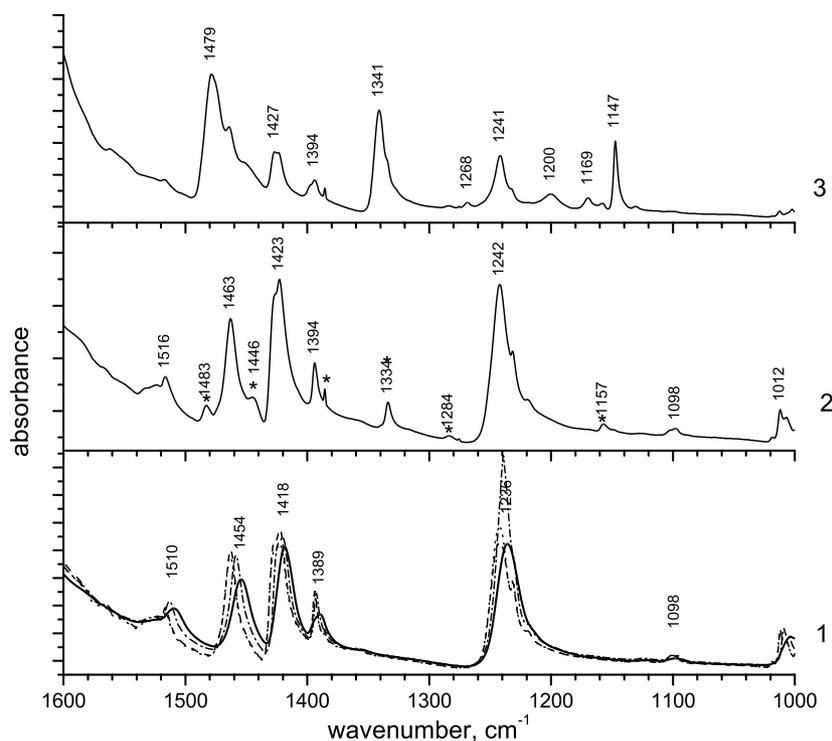

Fig. 4. FTIR spectra of uracil in the NH in-plane bending spectral region: 1–100% NH at 300 K—solid; 200 K—dash-dots; 100 K—dashes; and 10 K—dots; peak positions correspond to 300 K spectrum; 2–95% NH at 10 K; (∗) bands of doped ND groups; 3–15% NH at 10 K.

conditions and considerably reducing the amount of intensity transferred from the ν_1 (N3D) fundamental to the ND bending overtone, thus leading to the observed decrease in the intensity ratio.

As mentioned before, the band observed at 3088 cm^{-1} in the spectra of the 15% NH containing crystal corresponds to the CH antisymmetric stretching vibration. In the Raman spectrum of the uracil monomer isolated in an Ar matrix, the corresponding band was observed at 3084 cm^{-1} [15]. Note that in the IR spectrum of pure NH crystalline sample this band is observed only as a shoulder of the intense band at 3104 cm^{-1}, which integrates the complex band profile due to the coupled NH stretching vibrations (see Fig. 2A). The CH symmetric stretching mode (that has a larger contribution from C6H [12]) is here tentatively ascribed to the shoulder at 2985 cm^{-1}, which is clearly observed only at 10 K (see Fig. 3A, trace 2). The assignment of this mode has been the subject of great controversy in the past [12,15,21–23]. However, in consonance with the present assignment, in thymine, where due to the H-by-methyl substitution at the C5 position the coupling between the two individual CH stretching modes can no more operate, the CH stretching mode is observed at 3061 cm^{-1}, i.e., at nearly the average position of the two bands now assigned to the CH stretching modes in uracil.

3.1.1.2. Thymine. The spectra of polycrystalline NH and ND thymine obtained at 300 and 10 K (Fig. 5) are similar to the previously reported [18,26,29]. However, the NH/CH stretching region of isotopically diluted thymine cooled down to 10 K became considerably more informative than those of isotopically pure crystals. First of all, the band at 3061 cm^{-1} becomes well-resolved (Fig. 5B) and can be easily assigned to the C6H stretching mode, in agreement

with [29]. In addition, the bands at 2962 and 2932 cm^{-1}, which can be also clearly observed in the spectrum of the NH doped deuterated crystal and were found to be insensitive to the temperature change, can be now unequivocally assigned respectively to the antisymmetric (two modes) and symmetric stretching vibrations of the CH$_3$ group.

The band at 3008 cm^{-1}, which was not reported before—it becomes clear only at low temperature and for the isotopically diluted crystal (see Fig. 5)—is here assigned to one of the uncoupled NH stretching modes, while the band at 3040 cm^{-1} is ascribed to the second NH uncoupled stretching mode. Taking into consideration the calculated data reported in [22], the higher frequency band should be ascribed to the N1H stretching mode, whereas the lower frequency band shall be due to the N3H stretching vibration. The intense set of bands appearing above ca. 3100 cm^{-1} in the spectra of the crystals where NH containing species are dominant (see Fig. 5A), becomes weaker upon deuterium exchange (Fig. 5B) and results from the vibrational coupling involving NH groups taking part in multiple H-bonds and low frequency lattice vibrations. Indeed, when the concentration of NH groups in the sample is below ca. 10%, as in the case of the data shown in [29], these bands were not observed. In the spectra of the crystal where the concentration of the ND containing thymine is only ca. 5%, a doublet with components at 2306 and 2277 cm^{-1} can be clearly seen at 10 K (Fig. 5A, trace 3). This doublet reveals a clear temperature dependence (compare traces 2 and 3 in Fig. 5A) and its higher and lower components are assigned to N1D and N3D stretching modes, respectively. Note that this assignment leads to an isotopic frequency ratio for both N1H and N3H ν_1 modes of thymine close to 1.32, and is similar to that observed in uracil.

3.1.2. 1550–1000 cm^{-1} spectral range: the NH and CH in-plane bending vibrations

3.1.2.1. Uracil. The IR spectrum of the 100% NH uracil at different temperatures, in the 1550–1000 cm^{-1} spectral range, is shown in Fig. 4 (frame 1) together with that of the ND doped (trace 2) and NH doped (trace 3) crystals at 10 K. The data shown in Fig. 4 reveal that the spectrum of uracil in this spectral range is relatively simple at room temperature, showing essentially five bands. From those, only the weak band at 1394 cm^{-1} does not change its peak position and intensity with temperature and deuterium exchange. Its behavior upon temperature variation and isotopic substitution confirms its assignment to a skeletal ring or CH mode. On the other hand, the bands at 1516, 1463, 1423, and 1242 cm^{-1} (at 300 K) show significant blue shifts or/and narrowing at cooling, and become weaker at deuterium exchange; they shall be related to vibrational modes with relevant contribution of NH proton modes. In agreement with our conclusion, the calculations made in [21] for solid (15 K) uracil predicted that the PED contributions of NH modes to the 1516 and 1423 cm^{-1} bands should be ca. 30% (δN1H) and
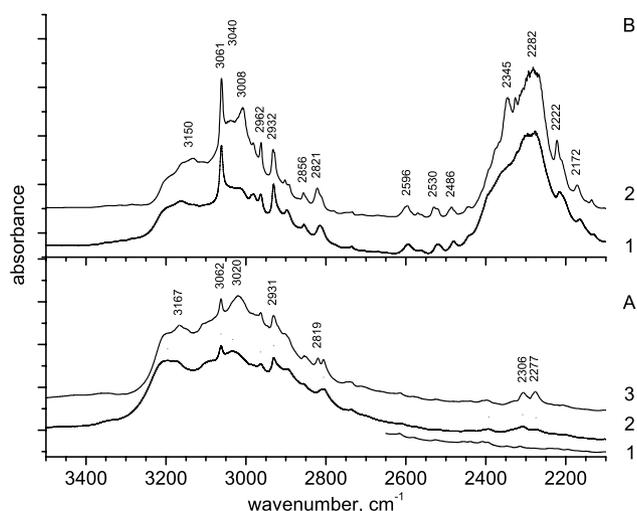
Fig. 5. FTIR spectra of thymine in the NH and CH stretching spectral region: (A) 1–100% NH at 10 K, for simplicity, only the absence of any bands below 2600 cm^{-1} is shown (above this frequency the spectrum is identical to that shown on trace 2); 2–95% NH at 300 K; 3–95% NH at 10 K; and (B) 1–20% NH at 300 K; 2–20% NH at 10 K.
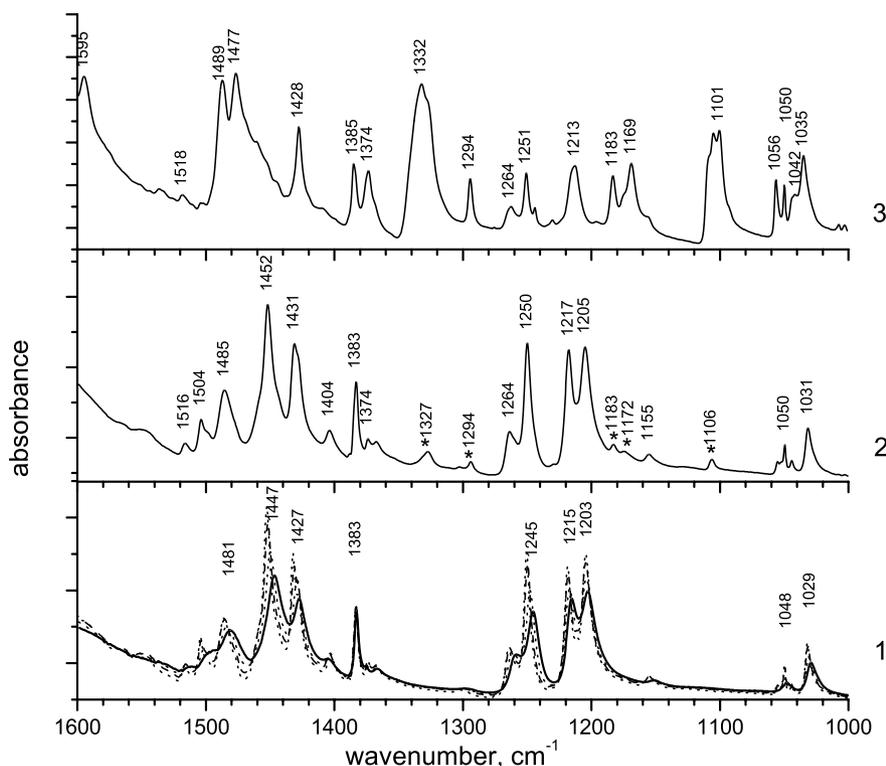
Fig. 6. FTIR spectra of thymine in the NH in-plane bending spectral region: 1–100% NH at 300 K—solid; 200 K—dash-dots; 100 K—dashes; and 10 K—dots; peak positions correspond to 300 K spectrum; 2–95% NH at 10 K; (∗) bands of doped ND groups; 3–20% NH at 10 K.

ca. 50% (δN3H). However, those calculations seem to underestimate the involvement of the NH in-plane bending coordinates to the vibrations that give rise to the bands at 1463 and 1242 cm^{-1}, predicting contributions of the NH in-plane bending coordinates for these vibrations below 6%.

3.1.2.2. Thymine. The 1550–1000 cm^{-1} spectral range of the IR spectrum of 100% NH thymine at different temperatures is presented in Fig. 6 (frame 1), together with those of the NH and ND doped samples at 10 K (traces 2 and 3, respectively). When used together, cooling and deuteration experiments reveal the bands that are not particularly sensitive to both temperature and isotopic changes. These are the weak bands observed at 1383, 1264, 1217, 1155, 1050, and 1031 cm^{-1} in the room temperature spectrum of the pure NH crystal. Their behavior confirms their assignment to CH$_3$ or CH bending or skeletal (ring stretching) modes [29]. The band at 1431 cm^{-1} is relatively more sensitive to temperature variation but it is not affected by deuteration and should also be assigned to a CH$_3$/CH bending or skeletal mode. All other bands show noticeable temperature changes and all of them almost disappear at D-exchange (compare traces 1 and 3 of Fig. 6). There are in the spectrum of NH-thymine at least seven bands, namely at 1516, 1504, 1485, 1452, 1404, 1250, and 1205 cm^{-1}, which are affected by cooling and D-exchange. In agreement with [29], we ascribe the intense band at 1452 cm^{-1} to a vibration with major contribution from the N1H in-plane bending coordi-

nate. The assignment of the remaining bands is more difficult, though all of them should have relevant contributions from the NH in-plane bending coordinates. In the spectrum of 5% ND doped thymine (Fig. 6, trace 2) new very weak bands due to vibrations of the ND groups appear at 1327, 1294, 1183, 1169, and 1106 cm^{-1}. The intensities of these new bands increase in the spectrum of the mostly ND-crystal (Fig. 6, trace 3), where additional new bands are also observed at 1489, 1477, 1374, 1056, and 1042 cm^{-1}. Among all those bands, that observed at 1169 cm^{-1} shows the most noticeable temperature narrowing effect and appears as the best candidate for being originated in the vibration with the largest contribution from the N3D in-plane bending coordinate. The corresponding vibration in the non-deuterated species should then be tentatively ascribed to the band observed at 1485 cm^{-1}, the isotopic frequency ratio for this mode being ca. 1.27 (the 1485 cm^{-1} band also exhibits the proton mode characteristic blue shift on cooling).

3.1.3. 1000 – 400 cm^{-1} spectral range: the NH out of plane bending vibrations

3.1.3.1. Uracil. The 1000–400 cm^{-1} region of the spectrum of pure NH uracil at different temperatures is presented in Fig. 7 (frame 1). The corresponding spectra of ND and NH doped samples are presented in the same figure (traces 2 and 3) for comparison. In general, in this region the spectra are similar to those previously measured [21,30].
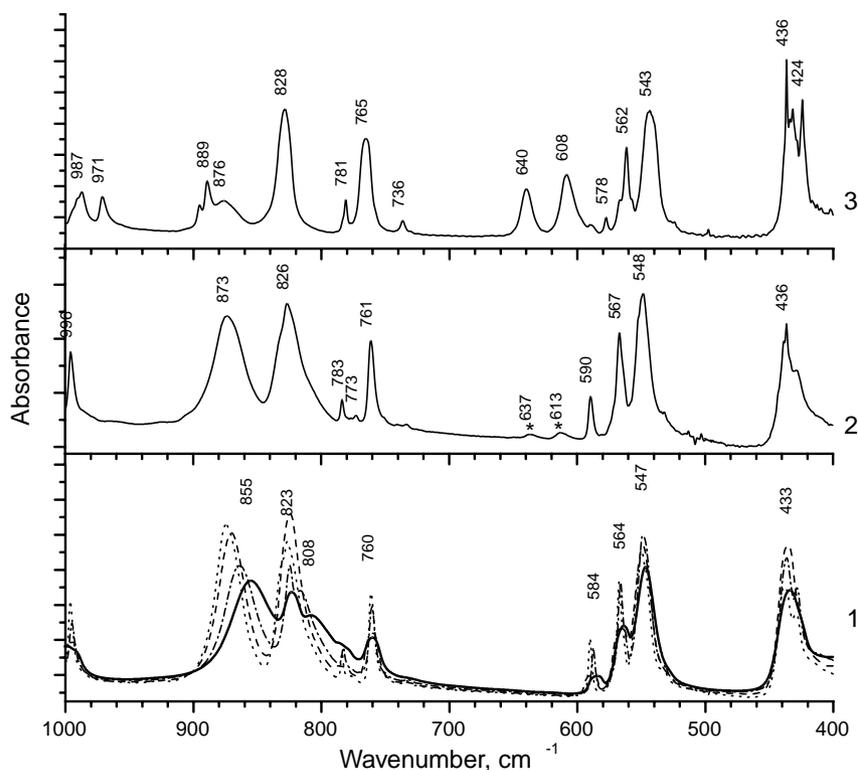
Fig. 7. FTIR spectra of uracil in the NH out of plane bending spectral region: 1—100% NH at 300 K—solid; 200 K—dash-dots; 100 K—dashes; and 10 K—dots; peak positions correspond to 300 K spectrum; 2—95% NH at 10 K; (∗) bands of doped ND groups; 3—15% NH at 10 K.

There are four bands, which are considerably affected by cooling and show isotopic shifts close to the expected. The most intense bands for the pure NH crystal are observed at 873 and 828 cm^{-1} at 10 K (at room temperature these bands overlap extensively). The remaining two bands are weaker and appear at 590 and 567 cm^{-1}. Note that the band observed at 808 cm^{-1} in the spectrum at 300 K undergoes a blue shift upon cooling and, at 10 K, it appears merged with the intense skeletal band at 826 cm^{-1} (Fig. 7, frame 1) The complex shape of the band at ca. 826 cm^{-1}, which results from the coalescence of the skeletal and ν_4 NH out of plane bending modes, can also be seen in the spectrum of the doped crystal where the amount of NH species is still ca. 95% (Fig. 7, trace 2). In this spectrum, as well as in the spectrum of the mostly ND containing crystal (Fig. 7, trace 3), the NH bands at 873 and 826 cm^{-1} (10 K) are related with their counterparts in the spectrum of ND-uracil appearing at 640 and 608 cm^{-1}, respectively (with isotopic frequency ratios of ca. 1.36, which are similar to those found for out of plane vibrations in cytosine [6]). On the other hand, the counterparts of the remaining two bands exhibiting both cooling and isotopic sensitivity could not be detected, since they must be of low intensity and are with all probability overlapped with the intense skeletal bands appearing at ca. 430 cm^{-1}.

The two intense bands can be unequivocally assigned to the out of plane ν_4 N3H (873 cm^{-1}) and N1H (ca. 828 cm^{-1}) modes, respectively, confirming the assignments

suggested in [31]. A more detailed examination of the 900–750 cm^{-1} spectral region, however, reveals peculiarities which were not reported hitherto. The first one is that the intensity ratio of the N3H and N1H ν_4 bands is ca. 2:1. Indeed, contrary to the ν_1 bands, whose intensity depends from their position (i.e., from the strength of the H-bond) and then their intensity ratios can only characterize the different proton ratios approximately, the intensity of the ν_4 bands were shown [3,4,32] to be independent of the H-bond strength and reflect the ratio of different protons in a crystal with accuracy not worse than 10%. Since X-ray structural methods suggested that both N1H and N3H should be each one involved in a single H-bond [9,10], we would then expect an intensity ratio of the N3H and N1H ν_4 bands of ca. 1:1. A possible explanation for this contradiction will be provided later in this paper. The second is the different intensity ratios of the ν_4 NH and ND bands, which is clearly shown in the spectra presented in Fig. 7. As mentioned before, in the 900–750 cm^{-1} range of the spectrum of the pure NH uracil, the band contour at 300 K (Fig. 7, frame 1; solid line) shows one relatively narrow band at 823 cm^{-1}, due to a skeletal mode, and at least two wide components at 855 (40, **34**) cm^{-1} and 808 (31, **15**) cm^{-1} (band widths and intensities, obtained by deconvolution are shown in parenthesis, the latter in bold type). The intensity ratio is ca. 2:1. The corresponding intensity ratio of the ν_4 bands in ND-uracil (appearing at 625 and 590 cm^{-1} at 300 K) is ca. 1:1. This value does not change at 10 K (see Fig. 7, trace 3).
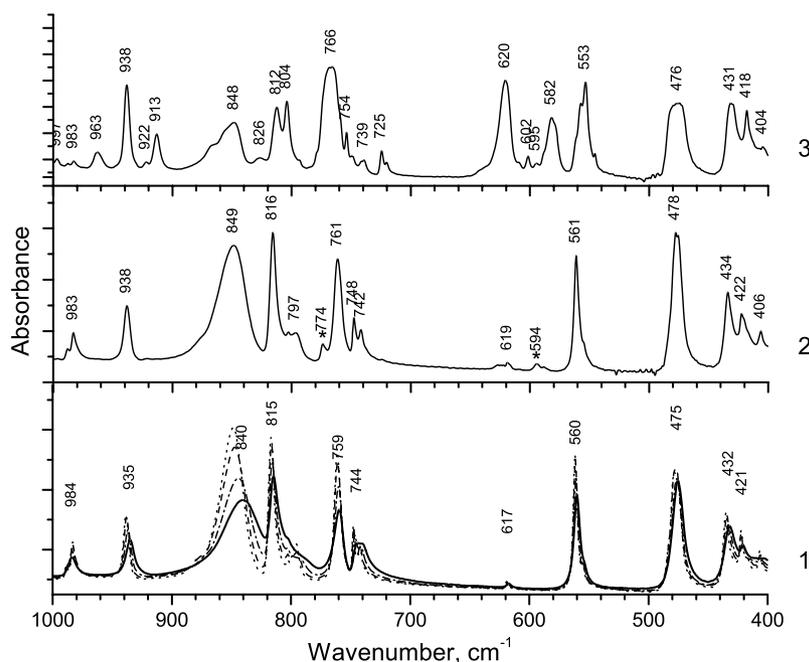
Fig. 8. FTIR spectra of thymine in the NH out of plane bending spectral region: 1—100% NH at 300 K—solid; 200 K—dash-dots; 100 K—dashes; and 10 K—dots; peak positions correspond to 300 K spectrum; 2—95% NH at 10 K; (∗) bands of doped ND groups; 3—20% NH at 10 K.

The structural changes occurring in uracil upon deuteration could be the most probable reason for different intensity ratio of the bands in NH- and ND-uracil (see below).

Other bands observed in the 1000–400 cm^{-1} region (see Fig. 7) do not show any remarkable changes with cooling and deuteration and should be assigned to skeletal modes.

3.1.3.2. Thymine. The spectra of all three samples of thymine are presented in Fig. 8 (traces 1, 2 and 3 belong to pure NH-, 95% NH- and 20% NH-samples, respectively) and, in general, are similar to those previously reported [23,30,33]. The weak bands at 983, 748, and 742 cm^{-1} and the relatively strong bands at 849 and 816 cm^{-1} (in the spectrum of the pure NH-sample obtained at 10 K) are significantly sensitive to cooling and D-exchange. The intensities of these bands fall off at deuterium exchange, and new bands appear at 725, 620, 582, 553, and 551 cm^{-1} in the spectrum of ND-thymine (compare traces 1 and 3 of Fig. 8). The isotopic frequency ratios of these bands are 1.36, 1.37, 1.40, 1.35, and 1.35, respectively. All these bands should have significant contributions from ν_4 NH (or ND) coordinates.

In consonance with [33], our results unequivocally show that the 849 cm^{-1} band should be ascribed to the ν_4 out of plane N3H bending mode. The assignment of the second expected ν_4 vibration was not straightforward, since the intensity of the corresponding band should not differ strongly from that of the 849 cm^{-1} band (the number of N1H and N3H groups is equal and in principle the intensity ratio expected for the ν_4 bands due to these two groups is 1:1). A closer inspection of the 900–780 cm^{-1} spectral multiplet

profile shows that, like in uracil, a relatively broad band centered at ca. 799 cm^{-1} is observed in the room temperature spectrum of thymine, with a relative intensity to the band at 842 cm^{-1} (which correlates with the band at 849 cm^{-1} in the spectrum at 10 K) equal to 1:2. Upon cooling, this band splits due to participation in resonant interactions; one component shifts to higher frequency, merging with the skeletal band observed at 814 cm^{-1} at room temperature, while the others give rise to the bands observed at 803 and 797 cm^{-1}.

The assignment of the ν_4 N3D and N1D bands could be made straightforwardly to the bands at 620 and 582 cm^{-1}, respectively; these two bands are narrow and well resolved and their assignment is absolutely clear. As in the case of the ν_4 NH bands, a ν_4 N3D/N1D intensity ratio of ca. 2:1 is observed for all temperatures studied.

Note that the nature of bands at 849 and 816 cm^{-1} was briefly discussed in [23] and these bands were there assigned to vibrations having only relatively modest contributions from the N1H and N3H out of plane coordinates (not more than 20–35% PED). In [29], the band at 849 cm^{-1} was considered to be the result of the overlapping bands due to the out of plane γ(C2=O) and γ(C4=O) modes, while the band appearing at 816 cm^{-1} was ascribed to a skeletal ring mode. Also the bands observed at 582 and 551 cm^{-1} in the spectrum of ND-thymine (that correlate with those at 816 and 742 cm^{-1} in the spectrum of the non-deuterated thymine which, as mentioned above, should have significant contributions from the out of plane NH proton modes) were also miss-assigned to skeletal modes [29]. Our assignment then confirms the experimental and calculation results reported

in [22] only in case of the ν_4 N3H band—its frequency was there estimated as being ca. 840–860 cm^{-1}.

The present assignments are in agreement with the temperature and isotopic substitution behavior observed for all these bands. In addition, the similarity in behavior found for thymine and uracil is also consistent with structural data that indicate that the H-bond interactions in the crystals of the two compounds are similar [9–11].

3.1.4. Summary of the main results extracted from the analysis of the spectra of uracil and thymine

1. The ν_1 bands of N1H and N3H groups in uracil are observed at 3106 and 3000 cm^{-1}, respectively (at 10 K). The corresponding N1D and N3D ν_1 bands in ND-uracil could not be assigned precisely, being tentatively ascribed to the main components of the band with complex contour observed in the 2370–2200 cm^{-1} region.
2. The two ν_4 bands of N3H and N1H protons in uracil are observed at 854 and 809 cm^{-1} at 300 K and at 873 and ca. 828 cm^{-1} at 10 K, respectively. The intensity ratio of these bands is ca. 2:1 at all temperatures studied. This intensity ratio differs from that observed for the corresponding ND ν_4 bands (which are observed respectively at 640 and 608 cm^{-1} in the spectra of ND-uracil at 10 K), that is approximately 1:1 and equal to that expected on the basis of the available structural information obtained from X-ray diffraction [9,10]. The different intensity ratio of the bands in NH- and ND-uracil suggests that structural changes occur in uracil crystal structure upon deuteration and/or that different resonant effects are operating in the two samples.
3. The previously reported red shifts in ν_1 [N1H (370 cm^{-1}) and N3H (375 cm^{-1})] and blue shifts in ν_4 [N1H (259 cm^{-1}) and N3H (231 cm^{-1})], which were assigned to closed uracil dimers and measured in Ar matrix [16] using as reference data the frequencies for the matrix-isolated monomer, are similar to those now measured in the cold crystal spectra (red shifts: 376 cm^{-1} N1H and 433 cm^{-1} N3H; blue shifts 257 cm^{-1} N1H and 191 cm^{-1} N3H). This result is a strong evidence of the structural importance of the dimeric units in the crystal of uracil, being in consonance with the available X-ray data [9,10].
4. In the thymine spectrum the N3H and N1H ν_1 bands are not resolved at 300 K, giving rise to the broad feature centered at 3024 cm^{-1}; it could be suggested that at 10 K the ν_1 bands of N1H and N3H protons correspond to the features observed at 3040 and 3008 cm^{-1}, respectively. The ν_1 bands of the N1D and N3D groups in deuterated thymine are observed at 2307 and 2271 cm^{-1} at 300 K and 2306 and 2277 cm^{-1} at 10 K. The contour of the main bands was found to be complex at 10 K. The isotopic frequency ratios for both vibrations were found to be ca. 1.32, indicating a relatively high anharmonicity for these modes.
5. The ν_4 bands of N3H and N1H protons of thymine are observed at 842 and ca. 799 cm^{-1}, respectively (at room temperature). At 10 K, ν_4 N3H is observed at 849 cm^{-1}, while ν_4 N1H gives rise to a complex band with components at 816, 803, and 797 cm^{-1}. In deuterated thymine the corresponding N3D and N1D ν_4 bands are observed (at 10 K) as narrow (ca. 10–11 cm^{-1}) and well resolved bands at 620 and 582 cm^{-1}. For both NH and ND crystals, the ν_4 N3H/N1H intensity ratio was found to be ca. 2:1; this intensity ratio differs from that expected on the basis of the available structural information, obtained from X-ray measurements [10,11].

The assignments here proposed for ν_4 vibrations in the two molecules studied deserve an additional note. It is well known that the ability of IR and Raman spectroscopies for examination of H-bond proton modes is considerably different, with the first method being much more sensitive to these vibrations than the latter. In general, scattering originating from H-bond proton modes is weak and very often the corresponding bands cannot be observed in Raman spectra. On the other hand, these vibrations give usually intense bands in infrared. Consequently, Raman spectra of condensed phases where H-bonds play an important role resemble more the Raman spectrum of the monomer than IR spectra of the condensed phase resemble the corresponding IR spectrum of the monomer. Indeed, as it was stated in [18], "Comparison of the Raman spectrum of thymine *isolated in the Ar* matrix with that of a *polycrystalline* sample indicates that they are quite similar, in marked contrast to the differences in the corresponding infrared spectra".

In consonance with the above information, all bands here ascribed to the ν_4 vibrations are very weak or absent in the Raman spectra of uracil and thymine crystals both at 15 K or at room temperature [21–23,29]. Furthermore, it can be noticed from the comparison of the Raman spectra of uracil and thymine at 15 K, presented in [21,23], that they are essentially different in the range below 1000 cm^{-1} (in all three spectral parameters: number of observed bands, peak positions and relative intensities), while the IR spectra of both solids are similar (compare traces 1 and 2 in Figs. 7 and 8). In particular, the most intense bands observed in this range for uracil (873, 827, and 590 cm^{-1}) and thymine (849, 816, and 561 cm^{-1}) have similar frequencies and sensitivity to both D-exchange and temperature variation (the relatively intense band at ca. 761 cm^{-1} in the spectrum of both compounds does not show the typical behavior of a proton mode regarding cooling and D-exchange—see Figs. 7 and 8—and should be assigned, in consonance with [29,33], to a skeletal mode. These results can only be explained if, as here considered, these intense IR bands that are absent (or very weak) in the Raman spectra belong to ν_4 modes of differently H-bond protons. The close peak positions of these bands in the spectra of the two compounds reflect the similarities of H–bonding interactions in the two crystals (and, consequently, of the energy associated with the different types of

H-bonds found in both crystals). Note that in consonance with this interpretation, the H-bond distances obtained by X-ray in both crystals are also very similar [9–11].

3.2. Spectral parameters and hydrogen bonding: use of empirical correlations

Once the assignment and accurate determination of peak positions of both ν_1 stretching and ν_4 out of plane bending NH mode bands could be undertaken, it is now possible to calculate the red shifts of ν_1 and blue shifts of ν_4 bands relatively to their position in the monomer and use these data to estimate their internal correlation and their correlation with the H-bond lengths and energies in the crystals of uracil and thymine; the latter results can be compared with independent thermodynamic data, since sublimation heats were previously measured experimentally for the studied crystals [34].

3.2.1. Prediction of the ν_4 bands positions from the red shift of ν_1: the abundance of different types of H-bond in uracil and thymine crystals

The calculation of the shifts in the position of both ν_1 and ν_4 bands in the crystal state is made relatively to their frequency in the monomers obtained from matrix isolation spectroscopy (ν^0). Several matrix isolation studies on uracil and thymine are available, but in all those studies the peak positions of the relevant bands agree within the experimental error. The data reported for the two molecules (Ar matrix) in [19,30] was here selected as reference data: for uracil, (N1H) group: $\nu_1^0 = 3482$ and $\nu_4^0 = 551\,cm^{-1}$; (N3H) group: $\nu_1^0 = 3433$ and $\nu_4^0 = 664\,cm^{-1}$; for thymine, (N1H) group: $\nu_1^0 = 3480$ and $\nu_4^0 = 545\,cm^{-1}$; (N3H) group: $\nu_1^0 = 3434$ and $\nu_4^0 = 662\,cm^{-1}$.

It was found [35] that the red shift of ν_1 and the blue shift of ν_4 bands associated with a given H-bond proton correlate to each other as $\Delta\nu_4^2 = 2.5\,(\Delta\nu_1)^{1/2} - 18$, where $\Delta\nu_4^2 \equiv 10^{-4}\,[(\nu_4)^2 - (\nu_4^0)^2]$ and $\Delta\nu_1$ is the red shift of the ν_1 band (both ν_1 and ν_4 are expressed in wavenumbers). Using this empirical relationship, the ν_4 frequencies in the crystal spectra of the two molecules were estimated from the observed red shifts of ν_1. For uracil, the N1H red shift is $\Delta\nu_1 = 376\,cm^{-1}$ and $\nu_4^0 = 551\,cm^{-1}$; thus the ν_4 (N1H) band in the uracil crystal, at 300 K, can be estimated to occur at ca. $780\,cm^{-1}$. Similarly, for the (N3H) group, $\Delta\nu_1 = 433\,cm^{-1}$, and the predicted position of ν_4 in the room temperature spectrum of the crystal is ca. $880\,cm^{-1}$. The estimated values are close to the two bands at observed at 808 and $855\,cm^{-1}$ at 300 K (873 and $828\,cm^{-1}$ at 10 K) and indirectly confirm the assignments made in the previous section for both ν_4 bands of N1H and N3H in the uracil crystal. Indeed, we can state that these two bands relate to the ν_1 modes of the N1H and N3H groups, which reflect the two main H-bond linkages present in the crystal, in agreement with the structural data obtained by X-ray [9,10]. The percentage

of these H-bonds is proportional to the contribution of the relative intensity of these bands to the total intensity associated with bands ascribed to ν_4 (which, as discussed before also include the bands observed at 590 and $567\,cm^{-1}$), i.e., ca. 82% (with relative accuracy of $\sim$15%). The observation of the bands at 590 and $567\,cm^{-1}$, which are also strongly sensitive to temperature variation and D-exchange, reflects the presence in the crystal of disordered (in the sense of not presenting long range periodicity) H-bonds. Such bonds, usually [1–7] are not observable in the ν_1 stretching range and cannot be detected by structural methods (e.g., X-ray) due to the insensitivity of these latter methods to short range interactions (in opposition to their ability to deal with long range periodic ordering). The relative intensities of the 590 and $567\,cm^{-1}$ bands to the total intensity associated with bands ascribed to ν_4 lead to an estimated amount of associated H-bonds of 5 and 13%, respectively.

A similar estimation for thymine can be made using the values extracted from the spectrum at 10 K for ν_1 (at room temperature, as described in the previous section, the two modes give rise to a single broad band centered at $3024\,cm^{-1}$): $3040\,cm^{-1}$ (N1H); $3008\,cm^{-1}$ (N3H). For this molecule, $\nu_1^0 = 3480$, $\nu_4^0 = 545$ and $\Delta\nu_1 = 440\,cm^{-1}$ (N1H), corresponding to a predicted position for ν_4 in the crystal of ca. $800\,cm^{-1}$; for N3H, $\nu_1^0 = 3434$, $\nu_4^0 = 662$ and $\Delta\nu_1 = 426\,cm^{-1}$, yielding a predicted frequency for ν_4 of ca. $880\,cm^{-1}$. The estimated frequencies for both N1H and N3H bands in the crystal are then in excellent agreement with the bands ascribed to these modes (ν_4 N1H: $799\,cm^{-1}$ and ν_4 N3H: $842\,cm^{-1}$, at room temperature; ν_4 N3H: $873\,cm^{-1}$, at 10 K).

The contribution (relative intensity) of the bands at 842 and ca. $799\,cm^{-1}$ to the total intensity associated with bands ascribed to ν_4 is ca. 84%. The other four bands in the thymine spectrum that are sensitive to temperature and deuteration (observed at 983, 748, and $742\,cm^{-1}$) correspond to relative intensities (i.e., abundances) of ca. 3, 8, and 5%, respectively, and are related to "disordered" H-bonds with different bond strengths.

3.2.2. Estimation of H-bond energies and H-bond contributions to sublimation heats in uracil and thymine crystals

It has been shown that the H-bond energy (in $kJ\,mol^{-1}$) and the red shift ($\Delta\nu_1$, in cm^{-1}) of ν_1 bands obey the correlation $(\Delta H)^2 = 1.92\,[(\Delta\nu_1) - 40]$ [28]. Using this relationship, the energies of the "ordered" H-bonds present in crystalline uracil and thymine can be estimated. For uracil, the calculated energies for the N1H and N3H groups $(-\Delta H)$ are 25.4 and $27.5\,kJ\,mol^{-1}$ per one proton, respectively. From the alternative empirical correlation between the H-bond energy and the blue shift of the ν_4 mode bands ($\Delta H = 0.67\Delta\nu_4^2$, where $\Delta\nu_4^2 \equiv 10^{-4}\,[(\nu_4)^2 - (\nu_4^0)^2]$, ν_4—in cm^{-1} [3,32]) these energies can also be estimated, being 23.4 and $19.4\,kJ\,mol^{-1}$, for N1H and N3H, respectively. For thymine the corresponding estimations are

necessarily less accurate due to larger uncertainties in the spectral data. For this molecule, the red shift correlation yields $-\Delta H = 27.7 \, \text{kJ mol}^{-1}$ for N1H and $27.2 \, \text{kJ mol}^{-1}$ for N3H, while the corresponding values extracted from the blue shift correlation are 22.9 and $18.1 \, \text{kJ mol}^{-1}$. Note that the values predicted on the basis of the two empirical correlations for N3H differ significantly from each other for both molecules, the observed differences being considerably larger than the expected error. Though no definitive explanation for this discrepancy can be provided here, it may result, at least in part, from use of overestimated reference values for ν_4^0 N3H. Indeed, these values were taken from matrix isolation data [19] and it is well known that under these experimental conditions specific matrix/solute interactions (e.g., those resulting from matrix packing constraints) might lead to increase the frequency of some out of plane bending vibrations relative to the free molecule.

The mean values for H-bond energies $(-\Delta H)$, calculated from the above data in uracil are ca. $24 \pm 5 \, \text{kJ mol}^{-1}$ for both N1H and N3H protons, while in thymine the corresponding values appear to be similar. These values are in reasonable agreement with independent thermodynamic data [34]—the binding energies of uracil and thymine dimers were measured as 19.8 and $18.8 \, \text{kJ mol}^{-1}$ per one H-bond, respectively, in the gas phase and, for uracil, $18.0 \, \text{kJ mol}^{-1}$ in an inert solvent [34]. Note that in the solid state, H-bonds are usually 20% stronger than in both the gaseous phase and inert solvent. The identical values of $-\Delta H$ in the crystals of the two compounds indicate similar H-bond networks and are also consistent with the already mentioned similarity of H-bond distances in both crystals [9–11].

Using a similar approach, the H-bond energies of "disordered" H-bonds could be estimated. For uracil, ν_4 bands at 590 and $567 \, \text{cm}^{-1}$, correlate with energies of ca. 3 and $1 \, \text{kJ mol}^{-1}$, respectively. In the case of thymine, the ν_4 bands at 983, 748, and $742 \, \text{cm}^{-1}$ correlate with energies of ca. 40, 13, and $12 \, \text{kJ mol}^{-1}$.

The above listed energies of all H-bonds present in the crystals, multiplied by their abundance, should reflect the H-bonding contribution to sublimation heats of uracil and thymine. From available experimental data [34] it can be concluded that the differences between the sublimation heats of uracil and *N,N*-dimethyl-uracil and between thymine and *N,N*-dimethyl-thymine (which should reflect the H-bonding contribution in uracil and thymine crystals, respectively) are ca. 29 and $15 \, \text{kJ mol}^{-1}$. If the crystals were ideal and contained only the types of H-bonds accounted by their X-ray structures [9–11], which, as estimated above, correspond to $-\Delta H \sim 24 \, \text{kJ mol}^{-1}$ (per bond), this contribution should be ca. $48 \, \text{kJ mol}^{-1}$. On the other hand, the estimated value obtained taking into consideration all types of H-bonds revealed by the analysis of the ν_4 spectral region in the present study is, for both compounds, ca. $35 \, \text{kJ mol}^{-1}$, thus improving the agreement with the experimental data obtained by an independent method [34]. These results give further support to our suggestion about the presence of additional

"disordered" types of H-bonds, with different energies, besides those probed by X-ray.

3.2.3. Possible explanation for the observed intensity ratio 2:1 for the ν_4 proton modes N3H/N1H in uracil and thymine and estimation of H-bond distances from the ν_1 red shift

As it was mentioned above, the presence of two different H-bonded protons connecting the units of the cyclic H-bonded dimers detected by X-ray in both uracil and thymine crystals, is expected to give rise to two infrared ν_4 bands (one associated to each group), which should have nearly equal intensities, since it is well known that the intensity of ν_4 mode bands is practically (within 10%) constant and, in particular, does not depend from the H-bond energy [1–7]. To the best of our knowledge, violation of this rule has been reported only once [13]: namely, it was mentioned that the intensity ratio of the N1H and N3H ν_4 bands might decrease for high concentrations of matrix isolated uracil, suggesting a different level of involvement of the two groups in the inter dimer association. This observation was not explained in [13] and we believe that the explanation might be connected with our observation that the ν_4 mode of the N1H proton gives rise to a less intense band than expected on the basis of the crystal structure obtained by X-ray. A possible reason for the above mentioned findings might be the involvement of the N1H proton in an additional H-bond. Indeed, the change of intensity can be correlated with a reduction of the effective charge at the proton, which, in turn, can occur if an additional electron donor is placed near the vibrating proton. The suggestion is that the N1H proton of one molecule participates in a "bifurcated" H-bond with two different carbonyl groups. The formation of the additional H-bond increases the frequency (ν) of the bending vibration of the proton and lessens its amplitude (x) [in a first approximation $x \propto (\nu)^{-1/2}$] and, consequently, the corresponding band intensity. This explanation can also account for the complex structure noticed in some of the proton bands, especially at low temperature, by increasing the probability of existence of slightly different local spatial arrangements leading to slightly different H-bonds. In uracil, for example, the N1H proton can establish a single H-bond with one O=C4 group (Fig. 9B) or, after a minor change in the interdimer distances, make an additional (bifurcated) H-bond with the O=C2 group of a neighboring molecule (Fig. 9A). Note that, in fact, the suggested re-arrangement of H-bonds practically does not change the positions of the heavy atoms, thus being compatible, within the experimental error, with the available X-ray data.

The disruption of described "bifurcated" structure can be a reason for intensity ratio change of the ν_4 N1H and N3H bands at deuterium substitution in uracil spectra (see above).

Another intriguing inconsistency between the structural data and the results extracted from the infrared spectra appears when a comparison is made between the H-bond distances reported in the X-ray studies [9–11] and those estimated using the empirical correlation between these
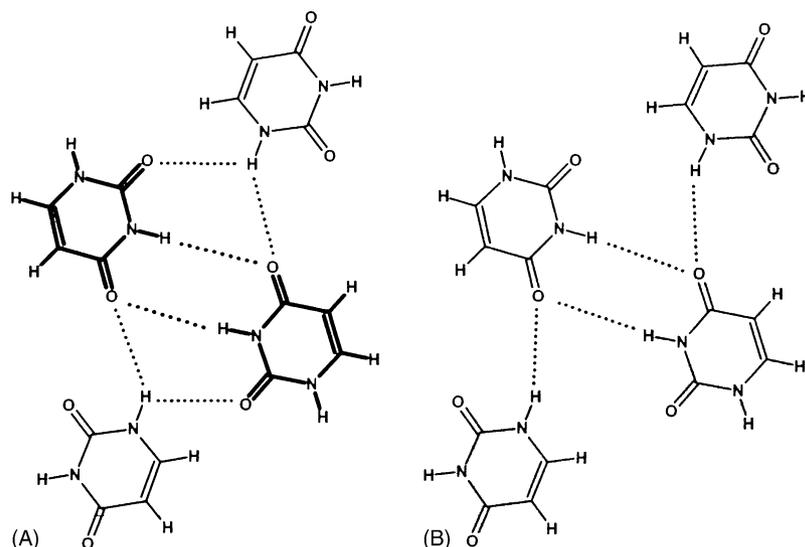
Fig. 9. (A) Possible alternative H-bond network for the same structural arrangement of uracil molecules, requiring only minor geometry changes in interdimer coordinates and (B) representation of the main structural unit of the uracil crystal, with H-bond network.

distances (r, in nanometer) and the observed ν_1 red shifts (in wavenumbers): $\Delta \nu_1 = 0.011 \, r^{-6.1}$ [1,2]. The dominant H-bond distances in uracil predicted using the red shifts/H-bond distance correlation (using the adequate temperature correction to the formula [4]) are 0.180 (H1···O4) and 0.176 nm (H3···O4). In the room temperature spectra of thymine only one red shift was measured and the corresponding (average) H-bond length is estimated as 0.181 nm.

On the other hand, using the data reported in the X-ray studies [9,10] (N···O distances, together with N3–H and N1–H bond lengths, the latter being respectively 0.088 and 0.084 nm) the H-bond lengths in uracil can be obtained: 0.202 nm (H1···O4) and 0.198 nm (H3···O4). For thymine, H1···O2, and H3···O2 H-bond lengths extracted from the X-ray data [10,11] are 0.202 and 0.193 nm, respectively.

When compared with the values estimated from the empirical correlation, the X-ray H-bond lengths are systematically larger by ca. 0.02 nm; this deviation is 4–5 times larger than the accuracy limits of the empirical correlation [2], which has been already successfully confirmed for more than 60 different hydrogen bonds. This discrepancy can, however, be solved if, instead of using the N–H bond lengths given in the X-ray studies (which, as usually for X-ray data should be taken with care due to the difficulty of the method to precisely determine the position of hydrogen atoms), a different approach is used to obtain the H-bond lengths. In computational studies dedicated to the analysis of the crystal packing in uracil and its derivatives, the so-called "standard" N–H bond length of 0.101 nm [36] was used. If this value is used together with the distances between the heavy atoms taken from the X-ray data [9–11], the H-bond lengths [r(H···O)] reduce to 0.185 nm for uracil, and to 0.182 (H1···O2) and 0.180 nm (H3···O2) for thymine, being practically identical (within the limits of the expected error of the correlation)

to the estimated values obtained from the red shift/H-bond distance correlation.

4. Conclusion

This study demonstrates the utility of combining isotopic exchange techniques and cooling to low temperature to extract detailed information on H-bonding properties from infrared analysis of both ν_1 and ν_4 proton spectral regions of crystals with complex H-bonding networks. For the first time, observation and assignment of the infrared bands due to different types of H-bonds present in the uracil and thymine crystals were undertaken. The bands of two imine proton stretching modes were identified in isotopically diluted samples at 10 K. The number of observed uncoupled ν_1 bands corresponds to the number of ordered H-bond contacts indicated by crystal structure analysis studies. However, our spectroscopic study reveals that the ordered contacts should represent only ca. 80–85% of the total amount of H–bonds in the two crystals, as follows from the analysis of the ν_4-mode spectral range. The remaining H-bonds correspond to "disordered" hydrogen bonds (in the sense of absence of the long range periodicity) that are not accessible to conventional structural methods such as X-ray diffraction. The H-bond energies estimated from empirical correlations from the infrared data agree well with independent thermodynamic experiments for cyclic dimers in both crystals and once again demonstrate the applicability of the empirical correlations here used to the study of complex H-bonded networks. The total contribution of all H-bonds to the sublimation enthalpy of both solids, estimated from the present infrared results, was also found to reasonably agree with independent experimental thermodynamic data. On the other hand, the inconsistencies detected between the

spectral and previously reported structural data, in particular those resulting from the detailed analysis of the ν_4 spectral range, led us to propose a structural model for the H-bond network in the two crystals, where, in addition to the two H-bonds revealed by X-ray experiments, additional types of H-bonds (including N1–H bifurcated ones) are considered; this model makes the spectroscopic and structural data consistent, improving the agreement between the two different sources of data.

Acknowledgements

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