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On the hydrogen bonding in uracil: its effect on the vibrational spectrum

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The effects of intermolecular hydrogen bonding on the vibrational frequencies of uracil are discussed on the basis of *ab initio* calculations. DFT methods are applied to calculated vibrational frequencies of various uracil dimers, namely six cyclic structures and four T-shaped arrangements, as well as eight uracil–water complexes. Frequency shifts experienced by some normal modes in the dimerisation are clearly correlated with acidity or basicity of the interaction sites. Interaction energies and frequency changes indicate that cooperativity plays a fundamental role in a self-association process. Moreover the cooperative effects increase their contribution with the strength of hydrogen bonding. The theoretical IR spectra of all the hydrogen bonded uracils considered here are compared with the FT-IR spectra of uracil and thymine measured in an N_2 matrix at different concentrations.

Introduction

Due to their biological importance, uracil (Fig. 1) and its derivatives have been investigated extensively by a variety of experimental techniques and theoretical methods. Much effort has been devoted to studying intermolecular hydrogen bonding. Vibrational spectroscopy has been one of the preferred techniques and infrared studies of molecules isolated in inert gas at low temperatures have been reported.[1–5] In recent years, *ab initio* MO theory has become an important tool for the interpretation of vibrational spectra and the assignment of the observed bands has been clarified by a series of theoretical studies and normal coordinate analyses.[6–9]

It is well known that the normal modes of uracil which involve stretching as well as in-plane and out-of-plane bendings of the C=O and NH groups undergo substantial changes in frequency due to hydrogen bonding.[10–12] Therefore, the frequency shifts of these modes can provide indirect information on the intermolecular interactions. For example, uracils showed a tendency to self-associate when the molecules were studied in concentrated and annealed matrices by FT-IR spectroscopy.[11] Similarly, the hydrogen bonding ability of several uracils towards water made it possible to isolate H_2O complexes in matrices and study their FT-IR spectra.[12]

However polyfunctional compounds such as uracil can form dimers of various structures depending on which site is involved in intermolecular interaction.[13–15] The complexity of the potential energy surface of gas-phase uracil dimer emerged from a recent theoretical study[15] where 11 minimum energy structures were located: seven cyclic and three open dimers as well as three structures describing stacked arrangements. The authors focused their attention mainly on the stabilities of dimers with accurate energy calculations up to the MP2/6-31G(d) level. The effects of hydrogen bonding on the molecular geometry of uracil have been the subject of an *ab initio* study by some of us[14] in which nine cyclic dimers were found to be structurally different. Thus it is expected that different patterns of aggregation could be spectroscopically distinguishable. However vibrational frequencies were not calculated at high levels in previous studies. A careful examination of wavenumber displacements from the monomer frequencies could instead give useful information on the most likely sites for hydrogen bonding and on the strength of the intermolecular interaction. For example, open dimers formed by only one C=O···H–N hydrogen bond, already taken into account to explain some FT-IR matrix spectra,[11] are of great interest to investigate the existence of a general correlation between the strength of the NH···O=C hydrogen bonding with the basicity and acidity of the respective interaction sites or to estimate cooperativity effects in the closed structures.

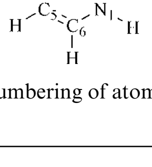

Fig. 1 Numbering of atoms in uracil.

The primary aim of the present work is therefore to investigate how various dimer arrangements can modify the vibrational spectrum of the uracil molecule. Some cyclic as well as open dimers are considered here and their vibrational frequencies are predicted by quantum mechanical methods using density functional theory (DFT). It has been demonstrated how such methods predict important properties (structure, dipole moments, energetics and vibrational frequencies) of isolated[16] and hydrogen bonded[17] molecules with similar accuracy to that obtained with the more expensive conventional correlated methods, MP*n*.[18]

The structures and vibrational spectra of uracil dimers also offer us the possibility of investigating the origin of the spectral modifications observed in a matrix with increasing

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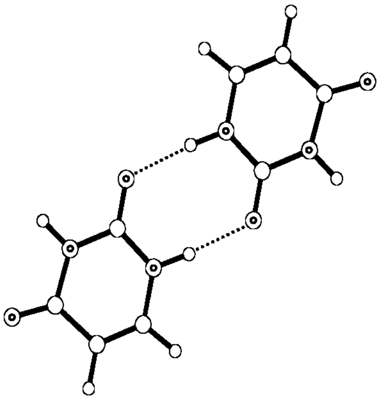


Fig. 2 The most stable structure (**2a**) of uracil cyclic dimers. Oxygen and nitrogen atoms are marked by circles. The hydrogen bonds are indicated by the dashed lines.

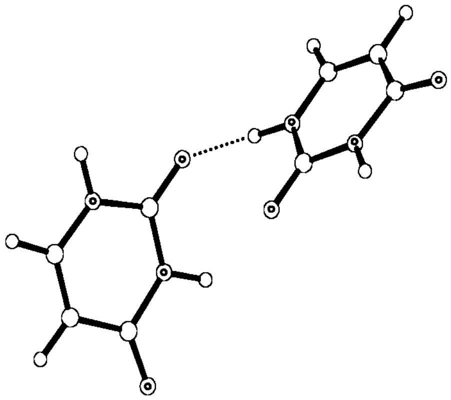


Fig. 3 A T-shaped uracil dimer. Oxygen and nitrogen atoms are marked by circles. The hydrogen bond is indicated by the dashed line.

concentration. The FT-IR spectra of uracil and its 5-methyl derivative, thymine, were thus measured in a nitrogen matrix at different concentrations and compared with the theoretical frequencies of different dimers in an attempt to identify which structures of the uracil dimer are really formed in a matrix.

For the purpose of comparison with dimers, water–uracil complexes, already the subject of previous theoretical studies,[19,20] were also investigated at the same level of calculations adopted for the dimers. The sizes of water–uracil complexes allow the application of higher levels of *ab initio* theory (up to MP2/6-311+G(d,p) level) to investigate how the values of frequency shifts change with the level of theory.

Computational methods

Ab initio molecular orbital calculations were run on an Alpha AXP-3000/500 cluster of CASPUR c/o CICS at the University of Rome using the GAUSSIAN 98[21] package.

The geometry and vibrational frequencies of the isolated molecule were obtained by the analytical based gradient technique within the C_s symmetry constraint. All the results were obtained using density functional theory, employing the BLYP (Becke exchange[22] and Lee, Yang, Parr correction[23]) and B3LYP (Becke's three parameter exchange[24] and Lee, Yang, Parr correlation[23]) potentials, and the MP2[18] level of perturbation theory combined with the 6-31G(d) and 6-311+G(d,p) basis sets.[25] Relaxation from planarity was allowed only at the MP2/6-311+G(d,p) level since at this level the C_s symmetry structure showed an imaginary frequency relative to an out-of-plane normal mode. However the deviations from planarity, expressed as displacement from the plane through C4C5C6 atoms, are indeed negligible (within 0.07 Å).

Two types of uracil dimers were considered here: six cyclic structures (Fig. 2) where two molecules interact through two $C{=}O\cdots H{-}N$ bonds and four open structures (Fig. 3) where molecules form only one $C{=}O\cdots H{-}N$ bond. All cyclic dimers were investigated under C_{2h} (cyclic structures) or C_s symmetry. No geometrical constraints were imposed to localise the stationary points corresponding to the four open structures. Frequency calculations revealed that such T-shaped structures, obtained from closed ones through rotations about one $C{=}O\cdots HN$ bond, are first-order saddle points whereas all cyclic dimers are minima. Frequency shifts from the monomer were calculated firstly for the most stable dimer at various levels, BLYP, B3LYP with the 6-31G(d) and 6-311+G(d,p) basis sets and MP2/6-31G(d), to evaluate the effect of the method of calculation, and then for all dimers at the BLYP/6-311+G(d,p) level.

The interaction of water with uracil was investigated through four complexes describing a double hydrogen bonding interaction ($C{=}O\cdots HO_w$, $NH\cdots O_wH$ or $C{=}O\cdots HO_w$, $CH\cdots O_wH$) and four complexes describing single $C{=}O\cdots HO_w$ or $NH\cdots O_wH$ interactions. Without any symmetry constraint, vibrational frequencies were obtained for the eight water complexes at the same level as that adopted for dimers, namely BLYP/6-311+G(d,p). As for dimers, the most stable complex was investigated at other levels of theory, B3LYP and MP2, with the 6-31G(d) and 6-311+G(d,p) basis sets.

An estimation of stability for the different hydrogen bonded structures was then obtained from the values of interaction energy. However, it must be stressed that no BSSE (basis set superposition error) corrections were accomplished since the main scope of the present work is not to investigate the stability of the complexes, already accurately studied in previous theoretical studies.[19,20]

Experimental

Commercial samples (Aldrich, 98%) were purified by vacuum sublimation prior to the experiment and vaporised at *ca.* 350 K from a small glass oven located inside the vacuum chamber. The vapours coming from the molecular source were mixed with nitrogen and deposited on a reflecting gold-plated copper cold-finger of a cryotip (202 CSA, Air Products and Chemicals) at 12 K. Spectra were recorded by reflections using a Bruker IFS 113v interferometer. 200 scans were accumulated at 1 cm^{-1} resolution. Different concentration matrices were obtained by regulating the temperature of vaporisation and the flow of the matrix gas. Cyclic annealing was repeated to encourage diffusion and association of monomer species.

Results and discussion

1 Uracil–water complexes

A preliminary study was carried out on the isolated molecule. Table 1 shows the unscaled harmonic vibrational frequencies of uracil obtained at the BLYP, B3LYP and MP2 levels with 6-31G(d) and 6-311+G(d,p) basis sets along with the experimental data measured in an argon matrix.[4] A comparison between theoretical and experimental frequencies reveals a substantial agreement at all the levels of calculations with scaling factors ranging from 0.93 to 0.99.

The interactions of uracil with water were then investigated by considering firstly the four complexes of Table 2. In all the structures, the water molecule is bonded to uracil through two hydrogen bonds ($C2{=}O\cdots HO_w$, $N1H\cdots O_wH$: **1a**; $C2{=}O\cdots HO_w$, $N3H\cdots O_wH$: **1b**; $C4{=}O\cdots HO_w$, $N3H\cdots O_wH$: **1c**; $C4{=}O\cdots HO_w$, $C5H\cdots O_wH$: **1d**) and one of the OH bonds of the water molecule points out of the plane of the uracil molecule. Accurate values of interaction energy[19]

Table 1 Vibrational frequencies (cm^{-1}) of free uracil molecule

No.	Major internal coordinates[a]	BLYP/ 6-31G(d)	BLYP/ 6-311+G(d,p)	B3LYP/ 6-31G(d)	B3LYP/ 6-311+G(d,p)	MP2/ 6-31G(d)	MP2/ 6-311+G(d,p)	Exp.[b]
ω_1	ν(N1–H)	3519	3531	3639	3638	3657	3665	3485
ω_2	ν(N3–H)	3479	3489	3603	3596	3613	3617	3435
ω_3	ν(C5–H)	3181	3163	3269	3243	3306	3283	
ω_4	ν(C4–H)	3140	3120	3228	3202	3265	3242	
ω_5	ν(C2=O)	1766	1721	1846	1803	1864	1823	1764
ω_6	ν(C4=O)	1724	1682	1811	1767	1822	1783	1706
ω_7	ν(C5=C6)	1623	1601	1692	1671	1710	1680	1643
ω_8	δ(N1–H)	1452	1441	1512	1499	1533	1514	1472
ω_9	δ(C6–H) + δ(N3–H) + δ(N1–H)	1381	1373	1430	1420	1447	1425	1400
ω_{10}	δ(N3–H) + δ(N1–H)	1349	1350	1413	1405	1436	1415	1389
ω_{11}	δ(C6–H) + δ(N3–H) + δ(N1–H) + δ(C5–H)	1329	1317	1390	1382	1411	1400	1359
ω_{12}	ν ring[c]	1200	1193	1238	1227	1270	1245	1217
ω_{13}	ν ring[c]	1134	1122	1202	1189	1236	1214	1185
ω_{14}	ν ring[c]	1051	1040	1095	1085	1112	1094	1075
ω_{15}	ν ring[c]	957	954	991	990	999	991	980
ω_{16}	ν ring[c]	924	920	969	964	989	978	958
ω_{17}	ν ring[c]	741	737	773	768	782	772	759
ω_{18}	δ ring	536	538	558	560	561	557	562
ω_{19}	δ ring	519	520	541	542	541	535	537
ω_{20}	δ ring	501	504	519	521	518	510	516
ω_{21}	δ(C=O)	367	371	384	388	383	385	391
ω_{22}	γ(C6–H)	916	926	968	971	920	933	987
ω_{23}	γ(C5–H)	778	780	812	812	787	779	804
ω_{24}	γ(C2=O)	706	713	752	755	725	722	757
ω_{25}	γ(C4=O)	699	697	730	725	686	687	718
ω_{26}	γ(N3–H)	661	654	690	673	709	657	662
ω_{27}	γ(N1–H)	532	538	561	555	559	535	551
ω_{28}	γ ring	376	379	395	393	371	357	411
ω_{29}	γ ring	163	157	170	163	159	141	185
ω_{30}	γ ring	135	134	149	142	133	118	

[a] ν = stretching, δ = in-plane bending, γ = out-of-plane deformation. [b] Ref. 4; Ar matrix spectrum. [c] Such modes show a strong mixing between stretching of ring bonds and in-plane bending of CH and NH groups.

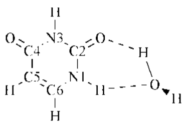
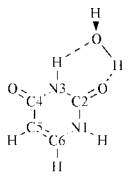
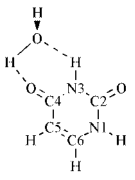
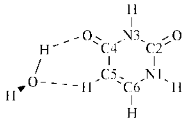
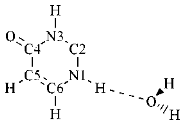
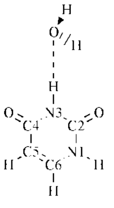
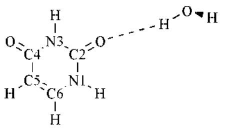
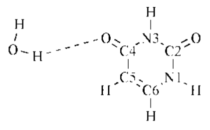
indicated that the structure **1a** is the most stable complex. Frequency shifts ($\Delta\omega$) for such an absolute minimum are strongly dependent on both the extension of the basis set and the level of theory. In particular we observe: i) the triple split valence 6-311+G(d,p) basis set predicts shifts (νN1H = −229, −210, −180 cm^{-1}; νC2=O = −22, −24, −22 cm^{-1}; γN1H = +248, +247, +312 cm^{-1}) smaller than those calculated with the 6-31G(d) basis set (νN1H = −358, −313, −245 cm^{-1}; νC2=O = −33, −43, −31 cm^{-1}; γN1H = +343, +321, +308 cm^{-1}) for all the levels employed (BLYP, B3LYP and MP2, respectively); ii) large differences are matched between the BLYP, B3LYP and MP2 values when the 6-31G(d) basis set is used whereas such values are more comparable on employing the 6-311+G(d,p) basis set. These results are not surprising since some care should be taken when adopting the small 6-31G(d) basis set in describing hydrogen bonded systems with DFT methods and the use of at least polarised-valence triple-zeta-quality basis sets augmented by diffuse functions such as 6-311+G(d,p) has been recommended.[17]

Comparison with the observed frequency shifts is not straightforward. Ar matrix spectra have shown that water forms both C=O···HO$_w$ and NH···O$_w$H bonds involving both the C=O groups.[12] However the existence of such closed water complexes was difficult to prove experimentally and only very recently *ab initio* calculations have explained the observed spectral changes with the formation of the **1a** and **1c** complexes.[20] On the basis of such an assignment, the observed shifts for the **1a** complex (Table 2) seem to be better reproduced when the 6-311+G(d,p) basis set is employed. Strong discrepancies with experiment still exist for the out-of-plane NH bending mode frequency. Such a large difference was interpreted in the previous study[20] as a probable erroneous assignment for this mode in the experimental study. Another point to be stressed is that the accuracy of properties of molecular complexes obtained from any supermolecular theoretical approach is always affected also by the BSSE. Due to this error, the computed interaction energies become too high and the potential energy surface distorted with respect to the relative experimental values. If BSSE corrections to the interaction energy can be done quite readily with the popular counterpoise (CP) method,[26] less attention has been devoted to the corrections to the intermolecular energy surfaces. From very small hydrogen bonded complexes it emerged that the frequencies of intermolecular modes generally decrease whereas those of intramolecular modes increase upon CP corrections although the extent of the effects largely depends on the complex as well as the level of calculation.[27] Unfortunately similar corrections were not evaluated for DFT methods.

The BLYP/6-311+G(d,p) level was chosen to consistently compare the wavenumber shifts of selected normal modes of uracil in the four cyclic water complexes. The downfield shifts calculated for the NH as well as C=O stretching vibrations are very close for all the complexes and perfectly comparable with the B3LYP/6-31++G(d,p) results of the previous study.[20] The values of $\Delta\omega$ for NH stretching modes are slightly higher for N1H (−229 cm^{-1}) than for N3H (−192 cm^{-1} and −214 cm^{-1}), reflecting the higher acidity of the N1H group. Similarly, such a different acidity is responsible for the higher blue-shift for the γ(N1H) out-of-plane vibration with respect to the corresponding γ(N3H) shift. The in-plane δ(NH) vibration contributes to several modes (for instance, ω_8, ω_9, ω_{10}, ω_{11}) and this makes it very hard to find a general correlation between the frequency shifts and acidity. Finally, the changes in the γ(C=O) vibrations are negligible.

To investigate the role of cooperativity resulting from cyclic arrangements of water a further four uracil–water complexes

Table 2 BLYP/6-311+G(d,p) wavenumber shifts ($\Delta\omega$) [cm^{-1}] of selected normal modes of uracil and interaction energy (ΔE) [kJ mol^{-1}] predicted upon water complex formation

	 **1a**	 **1b**	 **1c**	 **1d**
$\Delta\omega_1$ ν(N1–H)	−229 (−193)[a]	+2	−1	−2
$\Delta\omega_2$ ν(N3–H)	0	−192	−214	0
$\Delta\omega_5$ ν(C2=O)	−22 (−22)[a]	−28	+3	+4
$\Delta\omega_6$ ν(C4=O)	−1	+2	−23	−25
$\Delta\omega_8$ δ(N1–H)	+39	−4	+6	+2
$\Delta\omega_9$ δ(C5–H)	+7	−9	−7	+1
$\Delta\omega_{10}$ δ(N3–H)	−20	+60	+64	+5
$\Delta\omega_{11}$ δ(C6–H)	+40	+19	+7	+8
$\Delta\omega_{22}$ γ(C5–H)	+14	−2	0	+14
$\Delta\omega_{23}$ γ(C6–H)	+3	+1	0	+24
$\Delta\omega_{24}$ γ(C2=O)	+7	0	−2	+1
$\Delta\omega_{25}$ γ(C4=O)	−8	0	+4	+10
$\Delta\omega_{26}$ γ(N3–H)	−5	+165	+173	+3
$\Delta\omega_{27}$ γ(N1–H)	+248 (+159)[a]	+8	+12	+11
ΔE	41	32	35	27
	 **1e**	 **1f**	 **1g**	 **1h**
$\Delta\omega_1$ ν(N1–H)	−249	+1	−1	−4
$\Delta\omega_2$ ν(N3–H)	0	−215	−5	−2
$\Delta\omega_5$ ν(C2=O)	−13	−17	−8	+7
$\Delta\omega_6$ ν(C4=O)	−5	−3	+5	−17
$\Delta\omega_8$ δ(N1–H)	+43	−3	0	+5
$\Delta\omega_9$ δ(C5–H)	+5	−9	0	+1
$\Delta\omega_{10}$ δ(N3–H)	+5	+29	+1	+5
$\Delta\omega_{11}$ δ(C6–H)	+4	+13	+8	+4
$\Delta\omega_{26}$ γ(N3–H)	−8	+226	+1	0
$\Delta\omega_{27}$ γ(N1–H)	+325	+5	+6	+14
ΔE	31	21	16	22

[a] Assignment of the Ar matrix spectrum (ref. 12) based on the B3LPY/6-31++G(d,p) calculations of ref. 20.

were considered where only one hydrogen bond is taken into account. Such open structure complexes, indicated by **1e–h** in Table 2, are not minima on the potential energy surface, however they are suitable for investigating the effect of a single C=O···HO$_w$ or NH···O$_w$H interaction on the normal modes of uracil.

The following observations can be made from the data of Table 2.

i) Interaction energies of open structures indicate that more stable complexes are formed when more acid (N1H, **1e**) or more basic (C4=O, **1h**) groups are involved. The cyclic dimers are obviously more stable than open structures because of the formation of two hydrogen bonds, however no significant cooperative effect emerges from interaction energy values. All the cyclic structures in fact show a stability smaller than the sum of the interaction energies of two single hydrogen bonds as obtained from open dimers. Therefore each C=O···HO$_w$ and NH···O$_w$H interaction in cyclic arrangements is less stable than in open structures due to the fact that the formation of a six-membered ring can occur through hydrogen bonds less linear and with intermolecular H···O distances larger than open interaction.

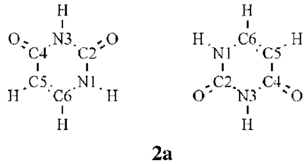
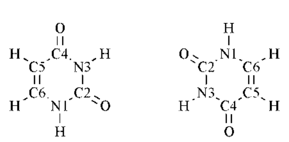
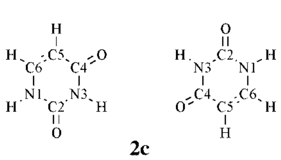
ii) The modes involving, almost 100%, the NH group, stretching and out-of-plane bending, are more highly shifted for open than for cyclic structures, reflecting the different strength of hydrogen bonding. The effect is slight for NH stretching but is dramatic for the γ(NH) mode.

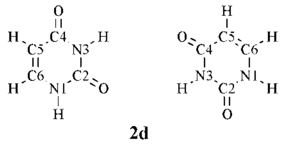
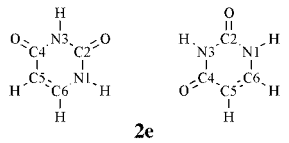
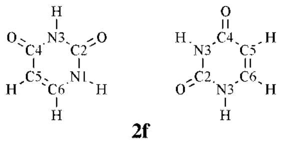
iii) The values of $\Delta\omega$ of both the C=O and NH stretching modes reflect the basic and acid power of the interacting sites. In contrast with cyclic complexes, the red shifts of C=O stretching modes are significantly different for the two C=O groups and a higher value is predicted for the group with the highest proton affinity, namely C4=O.[20]

2 Uracil dimers

Six closed dimers associated *via* two C=O···HN bonds were considered here. Consistently with previous MP2/6-31G(d) studies,[15] our BLYP/6-311+G(d,p) calculations indicate that the most stable dimer is the C_{2h} symmetry structure **2a** where a couple of C2=O···HN1 hydrogen bonds are formed (see Table 3). The order of stability of the remaining dimers is slightly affected by the level of calculation, however it is clear that more stable dimers are always formed when the hydrogen bonding

Table 3 BLYP/6-311+G(d,p) wavenumber shifts ($\Delta\omega$) [cm^{-1}] of selected normal modes of uracil and interaction energy (ΔE) [kJ mol^{-1}] predicted upon cyclic self-association in C_{2h} (**2a**, **2b** and **2c**)[a] and C_s symmetry (**2d**, **2e** and **2f**)[b] dimers

	 **2a**	 **2b**	 **2c**
$\Delta\omega_1\ \nu$(N1–H)	−373 (−420)	0	−2
$\Delta\omega_2\ \nu$(N3–H)	0	−245 (−273)	−307 (−344)
$\Delta\omega_5\ \nu$(C2=O)	−25	−21	+2
$\Delta\omega_6\ \nu$(C4=O)	−11	−4	−31
$\Delta\omega_8\ \delta$(N1–H)	+61	−2	+9
$\Delta\omega_9\ \delta$(C5–H)	+11	−9	−7
$\Delta\omega_{10}\ \delta$(N3–H)	+9	+70	+81
$\Delta\omega_{11}\ \delta$(C6–H)	+22	+19	+8
$\Delta\omega_{24}\ \gamma$(C2=O)	+7	−1	−2
$\Delta\omega_{25}\ \gamma$(C4=O)	−4	0	+3
$\Delta\omega_{26}\ \gamma$(N3–H)	−2	+212 (+185)	+239 (+212)
$\Delta\omega_{27}\ \gamma$(N1–H)	+330 (+295)	+7	+14
ΔE			
HF/4-31G(d)	66	37	39
MP2/6-31G(d)	67	45	44
BLYP/6-311+G(d,p)	66	37	42
R_1	1.179	1.156	1.167

	 **2d**		 **2e**		 **2f**	
$\Delta\omega_1\ \nu$(N1–H)	+2	−1	−414 (−399)	−5	−364 (−339)	+3
$\Delta\omega_2\ \nu$(N3–H)	−308 (−297)	−273 (−284)	−1	−326 (−340)	−1	−284 (−309)
$\Delta\omega_5\ \nu$(C2=O)	−32	+3	−45	+10	−22	−32
$\Delta\omega_6\ \nu$(C4=O)	−2	−30	+9	−41	−6	+7
$\Delta\omega_8\ \delta$(N1–H)	−3	−2	+58	+14	+55	+5
$\Delta\omega_9\ \delta$(C5–H)	−9	−8	+9	−6	+8	−9
$\Delta\omega_{10}\ \delta$(N3–H)	+69	+76	+9	+79	+9	+72
$\Delta\omega_{11}\ \delta$(C6–H)	+14	+7	+17	+8	+13	+37
$\Delta\omega_{24}\ \gamma$(C2=O)	0	−3	+7	−2	+6	+1
$\Delta\omega_{25}\ \gamma$(C4=O)	0	+3	−5	+3	+6	−1
$\Delta\omega_{26}\ \gamma$(N3–H)	+226 (+216)	+198 (+210)	+8	+229 (+222)	−7	+216 (+211)
$\Delta\omega_{27}\ \gamma$(N1–H)	+6	+14	+304 (+313)	+26	+282 (+289)	0
ΔE						
HF/4-31G(d)[c]	38		52		49	
MP2/6-31G(d)[d]	44		52		53	
BLYP/6-311+G(d,p)[e]	40		54		49	
R_1[f]	1.176		1.149		1.114	

[a] For the centrosymmetric dimers, frequency shifts are calculated for the B_u and A_u symmetry modes; in parentheses are shown the values relative to the IR inactive A_g and B_g modes. [b] In parentheses are reported the frequency shifts for the modes of one hydrogen bonded NH group calculated upon deuteration of the other interacting NH group of the second uracil molecule. [c] Ref. 14. [d] Ref. 15. [e] This study. [f] $R_1 = \Delta E_{\text{cyclic dimer}}/\Delta E_{\text{open dimer}_1} + \Delta E_{\text{open dimer}_2}$.

involves the N1H group (**2a**, **2e** and **2f**). As for uracil–water complexes, the values of frequency shifts were evaluated at different levels of calculation (BLYP, B3LYP and MP2), focusing attention on the most stable dimer (**2a**), but a less drastic dependence than that of the theoretical treatment was observed.

The vibrational spectrum of a uracil dimer consists of 66 normal mode frequencies; many of the intramolecular modes of the isolated molecule are recognisable in the complex. It is therefore easy to report the shifts experienced by these modes upon dimerisation (see Table 3). From a careful analysis of their values the following comments can be made.

i) The frequency shifts of all characteristic modes of uracil are clearly higher for a dimer association than for a water complexation.

ii) The NH stretching red shifts reflect the different acidity of the N1H and N3H groups better than those predicted for water complexes. The $\Delta\omega$ values for the N1H stretching mode in the different dimer structures, −373 cm^{-1} (**2a**), −414 cm^{-1} (**2e**) and −364 cm^{-1} (**2f**), are in fact drastically higher than those predicted for the N3H stretching mode, namely −245 cm^{-1} (**2b**), −307 cm^{-1} (**2c**), −308 and −273 cm^{-1} (**2d**), −326 cm^{-1} (**2e**) and −284 cm^{-1} (**2f**).

iii) The $\Delta\omega$ values for the **2a**, **2b** and **2c** cyclic dimers suggest that the C=O stretching modes suffer higher red shifts when the hydrogen bond involves the N1H group and consistently the C4=O group causes frequency displacement on the NH stretching higher than C2=O in harmony with the predicted higher proton acceptor power of the C4=O group.

These three important points indicate that there is a correlation between frequency shifts of such modes and acidity or basicity of the interaction sites. Further evidence of such a correlation is once again witnessed from the values of the blue-shifts for the γ(NH) out-of-plane vibrations which are significantly higher for N1H than for N3H.

3 Cooperativity in the strengthening of hydrogen bonding

C=O···HN hydrogen bonding is expected to be strengthened by cooperativity when cyclic dimers are formed. This mutual influence of the two hydrogen bonds on each other was previously detected by analysing the geometrical changes due to cyclic dimerisation and interpreted, in terms of valence bond theory, as an increase of importance of polar canonical forms $-C(-O^-)=NH^+$.[14] We can illustrate the point by considering the T-shaped structures (Fig. 3). The four different C=O···HN interactions were analysed through four open dimers. The salient points to be noted are summarised here.

i) The BLYP/6-311+G(d,p) interaction energies of the four open structure dimers (C4=O···HN3: −18 kJ mol^{-1}; C4=O···HN1: −31 kJ mol^{-1}; C2=O···HN3: −16 kJ mol^{-1}; C2=O···HN1: −28 kJ mol^{-1}) confirm that the strength of the intermolecular interaction is largely governed by the acidity of the NH group since stronger interactions occur through the more acid N1H group. The basicity of interaction sites has a minor effect and the C4=O forms dimers more stable by only 3 kJ mol^{-1} with respect to the C2=O group. Thus the strongest interaction occurs through the C4=O···HN1 bond and the weakest interaction through the C2=O···HN3.

ii) The values of interaction energies of cyclic and open dimers reveal the importance of cooperative effects in the self-association process which, on the contrary, were found to be absent in water complexation. The extent of such stabilisation is well suggested for example by the ratio (R_1) between the interaction energy of the cyclic dimer and the sum of the interaction energies of each C=O···HN hydrogen bond acting in the cyclic dimer as derived from open structures (see Table 3). The well recognised strengthening of intermolecular hydrogen bonding due to resonance, "resonance assisted hydrogen bonding",[28] is thus fully found in our results.

iii) The frequency shifts of all characteristic modes of uracil are higher for a cyclic structure than for an open self-association. As for stability, the extent of frequency shifts also clearly indicates that the formation of the second hydrogen bond reinforces the first one. A careful analysis of cooperativity from frequency shifts can be readily carried out only on isolated modes, such as the NH stretching and NH out-of-plane bending vibrations whereas it not easy for the C=O stretching modes. The latter ones are mutually coupled both in the monomer and in dimers and their real red-shifts can be masked. We first compared the C=O···HN interactions occurring in the centrosymmetric dimers with the analogous ones acting in open structures. We focused attention on the ratio (R_2) between the values of $\Delta\omega$ in cyclic and $\Delta\omega$ in open dimers both for the ν(NH) and γ(NH) modes relative to the group involved in hydrogen bonding. The R_2 values are not constant, as previously estimated on the basis of experimental frequencies,[11] but they reflect the strength of intermolecular interaction. As shown in Fig. 4, an interesting relation was in fact obtained between the values of R_2 and the difference between the proton affinities at the C=O site and deprotonation enthalpies of the NH site as obtained from B3LYP/6-31++G(d,p) calculations.[20] It appears therefore that the cooperative effect increases with the strength of hydrogen bonding. The same conclusion can be obtained by analysing a couple of different hydrogen bonds. Fig. 5 shows that the R_2 values for C2=O···HN3, the interaction matched four times in all the cyclic dimers, correlate with the nature of the second hydrogen bond. To minimise coupling effects between vibrations of NH groups of different molecules, the R_2 values were evaluated by considering the ν(NH) and γ(NH) frequencies of one group calculated upon deuteration of the second interacting NH group of the other uracil molecule. Fig. 5 shows that the mutual influence of the two hydrogen bonds on each other is not the same but increases with the strength of the intermolecular interaction.

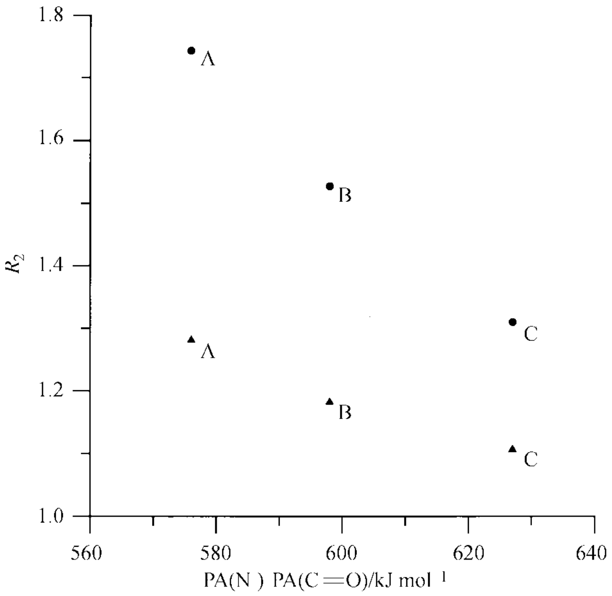


Fig. 4 Scatterplot of R_2 (ratio between $\Delta\omega$ in cyclic centrosymmetric dimers and $\Delta\omega$ in open dimers) *vs.* the difference between proton affinity of C=O, PA(C=O), and deprotonation enthalpy of NH, PA(N$^-$), of interaction sites. A: C2=O···HN1; B: C4=O···HN3; C: C2=O···HN3. Circles: ν(NH), triangles: γ(NH).

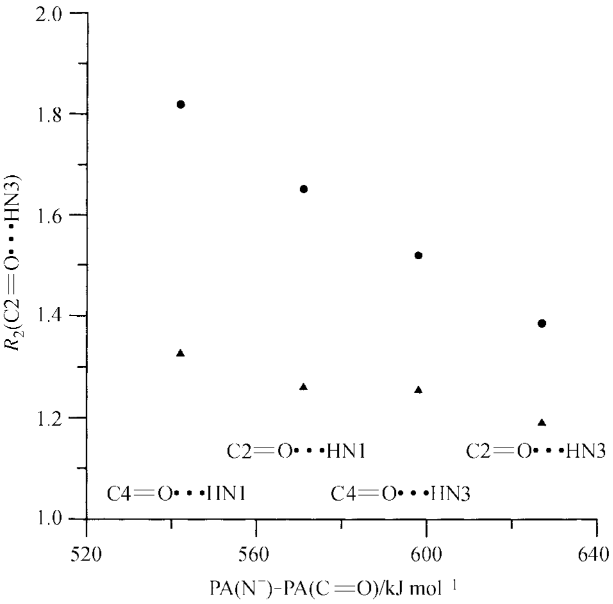


Fig. 5 Scatterplot of R_2 (ratio between $\Delta\omega$ in cyclic and $\Delta\omega$ in open dimers) for the C2=O···HN3 interaction *vs.* the difference between proton affinity of C=O, PA(C=O), and deprotonation enthalpy of NH, PA(N$^-$), relative to the second interaction. Circles: ν(NH), triangles: γ(NH).

4 Comparison with experimental data

The matrix spectra of uracil and thymine were measured in nitrogen matrices at different concentrations and after cyclic annealing. The spectral region of the NH stretching modes, and those of the NH in-plane (1500–1300 cm^{-1}) and the NH out-of-plane (1000–500 cm^{-1}) bending modes, are shown for both the molecules in Figs. 6–8. The spectral perturbations observed at high concentration are similar to those measured in an Ar matrix[11] and have already been interpreted in terms of formation of open and closed dimers. The results of our theoretical calculations now allow a better investigation of the structures of the dimers really formed in a matrix. Therefore Figs. 6–8 show the calculated spectra for all ten dimers as well as all eight water complexes previously described. The discussion is

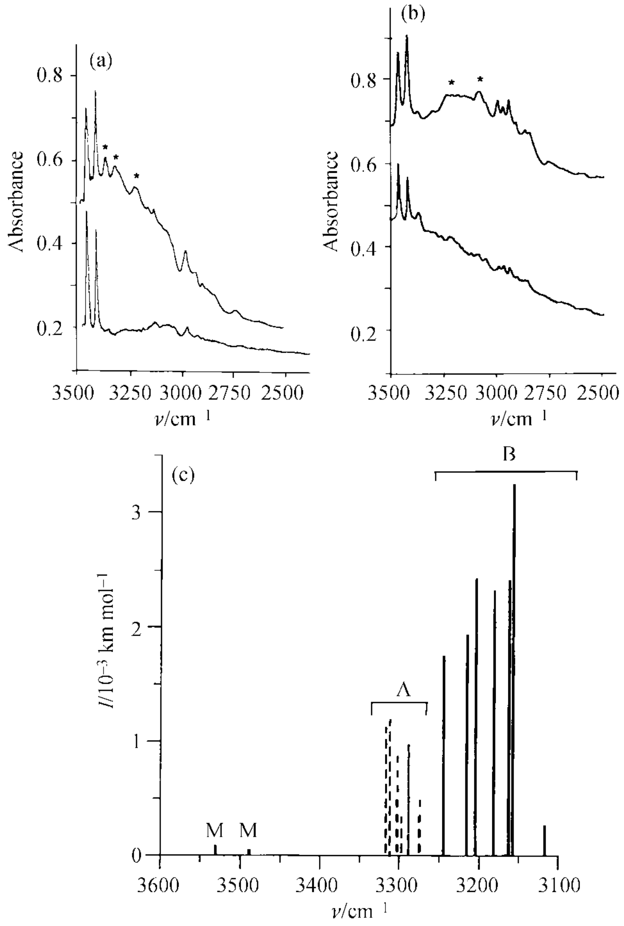


Fig. 6 FT-IR spectra in the ν(NH) region of uracil (a) and thymine (b) in dilute (bottom) and concentrated (top) nitrogen matrix (stars indicate dimer absorptions); (c) IR theoretical spectra of uracil calculated for monomer (M), water complexes (A), open dimers (A) and cyclic dimers (B).

organised separately for each spectral region. Notwithstanding the fact that the C=O stretching region is very sensitive to hydrogen bonding, it is well known that Fermi resonance causes a complication in the bands which can mask the spectral modifications caused by association in a matrix. For this reason we prefer to discuss only the spectral changes relative to NH vibrations.

1) Annealing induces the appearance of broad bands at about 3000 cm^{-1} both in uracil (Fig. 6a) and thymine (Fig. 6b) spectra which can be explained by the formation of dimers. Such a large downfield shift (*ca.* 300 cm^{-1}) is characteristic of cyclic dimerisation whereas smaller displacements from the NH stretching of the monomer are predicted for open dimers as well as water complexes (see Fig. 6). Therefore the presence of additional absorptions at 3300 and 3250 cm^{-1} indicates that uracil and thymine can also self-associate in a matrix through C=O · · · HN interactions weaker than those of closed dimers, for example by forming open structure dimers.

2) In the 1500–1300 cm^{-1} region we expect four NH in-plane bending modes (ω_8, ω_9, ω_{10} and ω_{11}) which are significantly sensitive to hydrogen bonding. However the relative absorptions for water complexes or open dimers are not manifestly different from those for cyclic dimers. The bands of this region instead reveal better than the NH stretching modes which NH group appears to participate in hydrogen bonding. This clearly emerges when we show separately the absorptions of uracil interacting through the N1H group (Fig. 7c) and those of uracil when the hydrogen bond involves the N3H group (Fig. 7d). The presence of bands at higher frequencies with respect to the ω_8 mode of the monomer, the N1H bending, is clear evidence of the participation of the N1H group in the intermolecular interaction. In contrast, interactions involving only the N3H group cause strong spectral perturbations only at frequencies lower than the ω_8 mode. The concentrated matrix spectra shown in Figs. 7a and 7b exhibit some bands of the monomer, ω_8 (1476 cm^{-1}), ω_9 (1405 cm^{-1}), ω_{10} (1389 cm^{-1}) and ω_{11} (1359 cm^{-1}) for uracil and ω_8 (1479 cm^{-1}), ω_9 (1417 cm^{-1}), ω_{10} (1389 cm^{-1}) and ω_{11} (1355 cm^{-1}) for thymine along with new absorptions, the intensity of which increase with matrix concentration. In the thymine spectrum one of these new bands (1485 cm^{-1}) appears only as a shoulder of the N1H bending absorption of the monomer and this could exclude the participation of this group in intermolecular interactions, at least under moderate association conditions. Such a small upfield shift for the N1H bending mode could be indirectly caused by the intermolecular interactions involving the N3H group (see Fig. 7d). On the other hand the formation of C=O · · · HN3 hydrogen bonding is well witnessed by the broad bands at 1420 cm^{-1} (uracil) and 1438 cm^{-1} (thymine), consistent with the theoretical spectra of Fig. 7d. Further evidence is found from 1-methyluracil which forms only C=O · · · HN3 hydrogen bonding and shows such a mode at 1438 cm^{-1}.[11] Also the uracil spectrum exhibits a broad band at higher frequency with respect to the N1H mode of the monomer (*ca.* 1500 cm^{-1}), however the entity of this upfield shift is clearly higher than for thymine, thus suggesting the formation of C=O · · · HN1 interactions.

3) The region below 1000 cm^{-1} is typical of the γ(NH) modes and Fig. 8 shows separately the γ(NH) bands predicted upon cyclic dimerisations (Fig. 8d) and those upon weaker intermolecular association (open dimers and water complexes) (Fig. 8c). As for NH stretching, the γ(NH) blue-shifts are fully representative of the strength of intermolecular interaction: all the cyclic dimers considered here show γ(NH) bending modes at a frequency very similar and close to 900 cm^{-1}; an open dimerisation or a water complexation is expected to be the origin of absorptions spread around *ca.* 800 cm^{-1}. In addition, the theoretical results indicate that it is very hard to identify which NH group is really involved in intermolecular association since the γ(NH) frequencies of hydrogen bonded uracils are very close both for C=O · · · HN1 and C=O · · · HN3 interactions. The formation of dimers in a concentrated matrix is well witnessed for uracil by the appearance of the broad band at *ca.* 900 cm^{-1} (Fig. 8a) which is characteristic of cyclic dimerisation; in contrast the moderate association conditions obtained upon matrix annealing do not cause appreciable changes in the spectrum of thymine (Fig. 8b). The only clear spectral modification concerns the relative intensities of the γ(N1H) and γ(N3H) bands: the latter manifestly decreases at higher concentration suggesting a greater participation of the N3H group in intermolecular association.

It is not easy to explain the origin of these spectral differences for two molecules structurally very similar like thymine and uracil. However we can derive some important conclusions from the analysis of their spectra. Firstly we can reasonably exclude that the dimer observed from the matrix spectrum is formed during the vaporisation process and then before matrix deposition since its structure should show the same hydrogen bonds which the molecules form in the respective crystals. This clearly appears for thymine where the lack of spectral evidence for the formation of C=O · · · HN1 interactions clearly contrasts with the crystal structure where each molecule forms hydrogen bonds through both the N1H and N3H groups.[14] Uracil crystal consists of centrosymmetric dimers with C4=O · · · HN3 bonds connected by additional bifurcated C2=O · · · HN1 interactions.[29] Thus a dimer formed directly during the vaporisation process could have the centrosymmetric structure **2c**; any other structure with at least one C=O · · · HN1 hydrogen bond should be excluded. Alternatively, we should consider the formation of less stable structures where monomers are coupled by C4=O · · · HN1 and C2=O · · · HC5 bonds.[14]

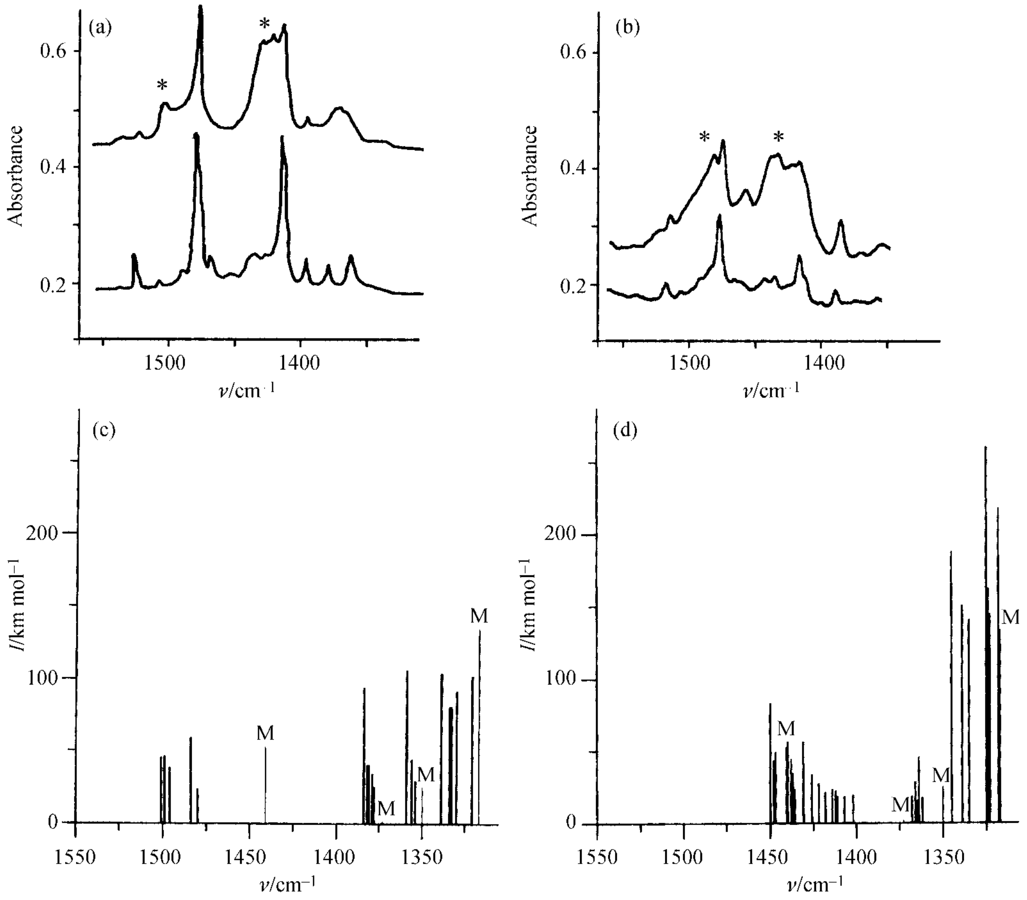


Fig. 7 FT-IR spectra in the δ(NH) region of uracil (a) and thymine (b); δ(NH) absorptions calculated for the monomer (M) and for a uracil molecule hydrogen bonded *via* the N1H group (c) or *via* the N3H group (d).

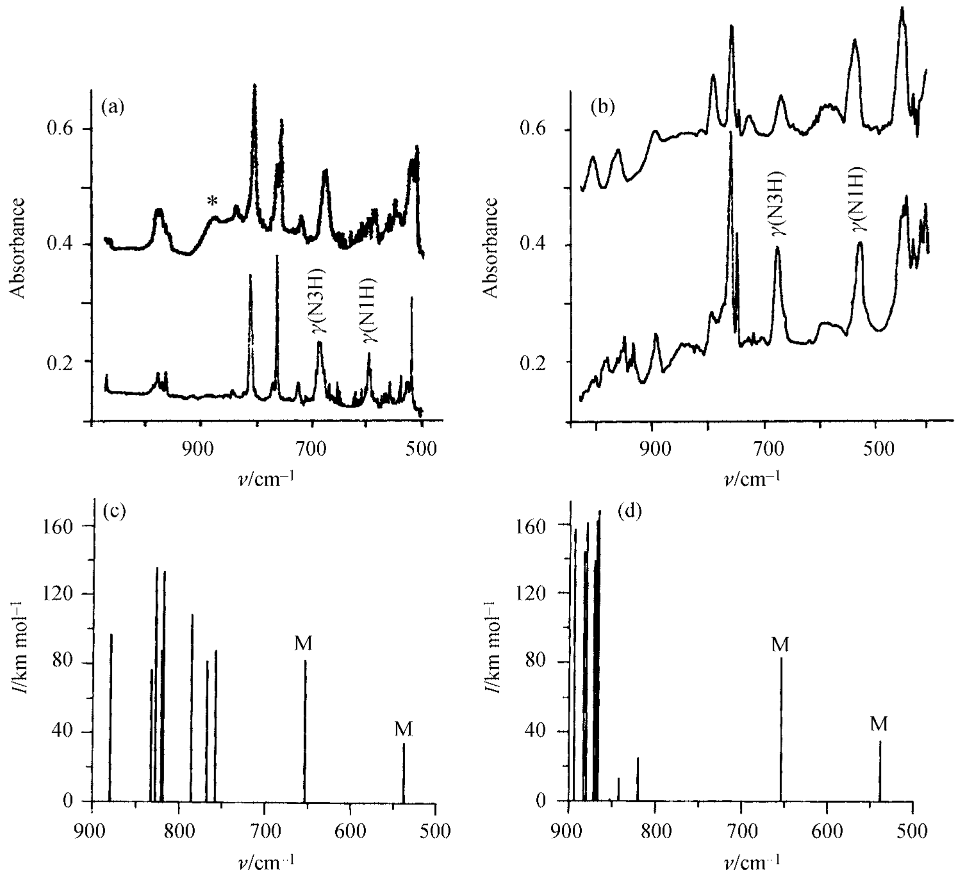


Fig. 8 FT-IR spectra in the γ(NH) region of uracil (a) and thymine (b); γ(NH) absorptions calculated for monomer (M), water complexes and open dimers (c) and cyclic dimers (d).

The results for thymine convinced us that dimerisation indeed occurs in a matrix upon monomer diffusion and in principle can result in the simultaneous formation of different structures. From stability considerations, there are no drastic preferences for any structure to exclude different dimers from coexisting in a matrix experiment. By leaving out any stability consideration, we could consider the simultaneous formation of all the six cyclic dimers and observe that the C=O · · · H3N bond is formed eight times whereas the C=O · · · HN1 bond is formed only four times. Therefore we expect to measure in the uracil spectrum the changes caused by C=O · · · H1N as well as C=O · · · H3N hydrogen bonds. The latter are more outstanding because of the higher number of uracil molecules involved in the C=O · · · HN3 interaction. The weight of each hydrogen bond in the resulting spectrum is then determined by the relative stability of each dimeric structure. The small increases in acidity of the N3H group as well as in basicity of the C2=O group calculated for thymine[20] could change the order of stability of dimers found for uracil and favour those structures where the C2=O · · · HN3 bond is formed. As a consequence, the spectral modifications typical of such an interaction are expected to increase in importance with respect to those characteristic of C=O · · · HN1, consistent with the experimental evidence for thymine.

Conclusions

There is a correlation between acidity or basicity of the interaction sites and the values of frequency shifts observed for those normal modes involving vibrations of hydrogen bonded groups. Such an important point clearly emerges from the study of cyclic dimers whereas it is not entirely found from the examination of water complexes. Cooperativity plays a fundamental role in a self-association process of uracil, increasing its contribution with the strength of intermolecular interaction and following the order of stability found for the four types of hydrogen bonding, namely C2=O · · · N3H, C4=O · · · N3H, C2=O · · · HN1, C4=O · · · HN1.

Although it is very hard to identify the structure of hydrogen bonded complexes really formed in a matrix with increasing concentration some differences are observed between uracil and thymine. C=O · · · HN3 hydrogen bonding is the main cause of intermolecular interaction for thymine, at least under conditions of moderate association. In contrast, both C=O · · · HN1 and C=O · · · HN3 interactions are found to operate in the formation of uracil complexes.

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