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AB Initio Calculations and IR Studies of Tautometric forms of Uracil and Cytosine and comparing results in different temperatures (25˚C, 37˚C and 40˚C).

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ABSTRACT

In this paper, the molecular geometry for three tautomers of uracil and four tautomers of cytosine has been analyzed. vibrational IR spectra of the tautomers were investigated at HF and B3LYP level using the AB initio 6-31G* and LANL2DZ basis sets from the program package Gaussian 98 (A.7 Public Domain version).
The physico-chemical and biochemical properties of uracil and cytosine are one of the principal criteria for the selection of these compounds. Calculated results were compared with the corresponding experimental data if available. The harmonic wave numbers for the main tautomers of uracil (U_1) and cytosine (C_2) will compare at the 25ºC, 37ºC, and 40ºC.

Keywords: tautomer; uracil and cytosine; ab initio calculations; DFT; HF; frequency

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INTRODUCTION

An understanding of the physiochemical properties and tautometric behavior of the purine and pyrimidine bases of the nucleic acids is of fundamental importance not only in relation to qualitative concepts of chemical binding and physical chemistry but also in relation to molecular biology. Particularly, several structural features that is necessary for the biological functions of nucleic acids, such as DNA double helix formation or RNA folding.

Today, quantum chemistry is almost universally applicable to the interpretation of physical and chemical properties of compounds. On the other hand, this science has been very successful in predictions of a broad range of ground state properties. Very recently several papers have been published which attempt to predict, with inclusion of electron correlation by density functional theory (DFT), the molecular parameters of the RNA bases [1, 2].

Uracil is one of the building pyrimidine nucleobases of RNA.In DNA, it is replaced by cytosine. The three uracil tautomers and the four tautomers of cytosine are the subject of studies which perform in ab initio and DFT levels of theory.

The aim of the present paper is 3 fold :

1.We discuss the properties and the stabilities of the tautomeric species of uracil and cytosine to compare these data with recent experimental data.

2.Calculation of the harmonic wave numbers and absolute intensities by DFT and HF levels for all tautomers, and comparison with the recorded IR spectra of these compounds in argon and nitrogen matrixes. For a better comparison of the predicted harmonic wave numbers with recorded wave numbers of fundamental modes, we scaled the predicted wave numbers by 0.91 and 0.89 in the case of DFT and HF calculations, respectively .

3.Investigations of the harmonic wave numbers calculated by DFT and HF levels for the main tautomers of cytosine (C2) and uracil (U1) in 37ºC and 40ºC which are compared with the results in 25ºC.

Computational and Theoretical Methods

Two quantum-mechanical approaches were used in this study:

Because the role of individual energy contributions for uracil and cytosine, it would be describe to investigate the process by means of a method which includes all interaction energy terms. This task can be achieved only by ab initio quantum mechanical (QM) calculations with the inclusion of electron correlation effects .

a)Conventional ab initio calculations at the Hartree-Fock (HF) level and,

b)Density functional theory (DFT) with the B3LYP level.

In all calculations the 6-31G* basis set was used for all atoms supplemented with a set of six d and three p polarization functions on heavy and hydrogen atoms, respectively. All geometries were fully optimized and calculated harmonic wave numbers scaled by factors of 0.91 and 0.89 for the DFT and HF calculations, respectively. All calculations were performed using the Gaussian 98 suit of packages.

RESULTS AND DISCUSSION

1)Optimized Geometries of tautomer forms.

This work describes the performance of quantum mechanical and theoretical method in calculating the energies, bond length, angle bond and vibrational frequencies of tautomeric forms of uracil and cytosine.

The strength of binding usually correlates with the molecule's biological activity, and several energy contributions may be responsible for the binding.

Nevertheless, it has been known for a long time that uracil and cytosine may also in tautomeric forms, whose activation barrier control their formation and determined actually their relative populations. These have been the subject of many experimental and theoretical studies in the past [3-6.[

The three optimized structures of uracil and four optimized structures of cytosine displayed in Fig.l.

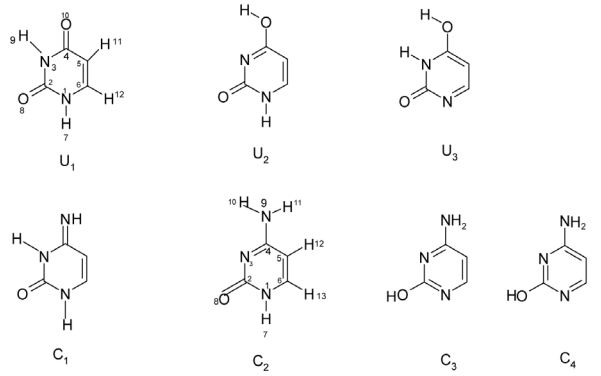
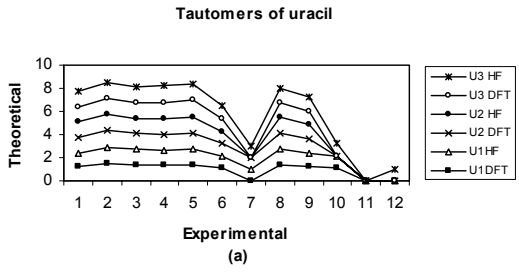


Fig.1. Optimized tautomers of uracil and cytosine with ab initio calculations.

Their optimized geometries are collected in Tables 1-2.In the tautomers of uracil, the C4 – O10 bond in the keto-enol forms is elongated 0.12Å in DFT (0.13Å in HF) versus the diketo form. For the two keto-enol structures of uracil, all of the intra-ring distances, with the exception of the C5 – C6 bond and the C2 – N3 bond only in the U3, become smaller. This may be accounted for by the increasing aromatic character of the six-membered ring.

The C4 – N9 bond in the amino forms of cytosine is elongated 0.04 – 0.08Å in DFT method (0.09 – 0.10Å in HF) versus the imino form. The N3C2O8 bond angle in the enol form of the U3 and the oxo-amino form of the C4 decrease with respect to other tautomers. To assess the accuracy of the geometries reported here our optimal bond lengths and angles of U1 and C2 are compared in Tables 1-2 with the average ring structures obtained from X-ray studies [7, 8].

Considering Fig.2. show that B3LYP/6-31G*optimized geometries of uracil and cytosine are closer to experimental results than HF/6-31G* optimized data.U1 tautomer of uracil and C2 tautomer of cytosine are more stable than other tautomers of these indicated compounds.



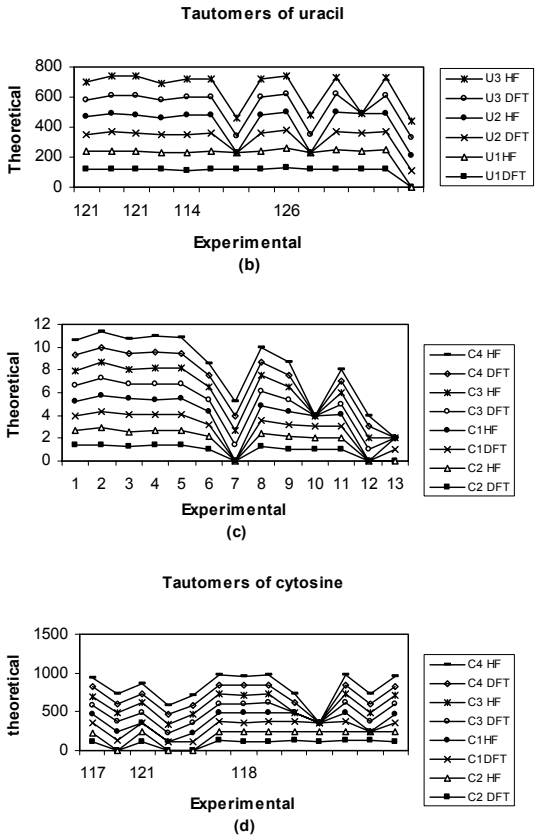


Fig.2. Comparison of bond lengths and bond angles of uracil (U1,U2,U3) **(a),(b)** and cytosine (C1,C2,C3,C4**) (c),(d)** tautomers with ab initio calculations at HF and DFT to experimental methods.

Table1. Optimized geometries of the tautomers of uracil.

Geometry	U1	U2	U3	Exp.
		Bond length (Å)		
C4-O10	1.210a(1.193)b	1.343(1.318)	1.343(1.321)	1.233
C5-C4	1.460(1.463)	1.430(1.434)	1.374(1.354)	----------
N3-C4	1.413(1.390)	1.307(1.282)	1.354(1.345)	1.380
C6-C5	1.349(1.328)	1.360(1.339)	1.414(1.416)	1.343
C2-N3	1.384(1.370)	1.380(1.370)	1.443(1.411)	1.381
H11-C5	1.080(1.070)	1.080(1.069)	1.080(1.069)	----------
H9-N3	1.012(0.997)	----------	1.013(0.996)	----------
N1-C6	1.375(1.372)	1.356(1.351)	1.314(1.289)	1.370
O8-C2	1.216(1.193)	1.216(1.193)	1.215(1.192)	1.219
H1-2C6	1.084(1.073)	1.084(1.073)	1.090(1.078)	----------
H7-N1	1.009(0.993)	1.011(0.994)	----------	----------
HO10	----------	0.972(0.948)	0.967(0.944)	----------
		Bond Angle(D)		
O10-C-4C5	126.2(125.5)	116.3(115.9)	121.4(122.1)	----------
C4-C5-C6	119.9(119.2)	115.1(114.7)	115.9(115.2)	120.7
C6-C5-H11	121.9(122.4)	123.1(123.7)	123.0(123.0)	----------
C5-C6-N1	121.8(122.2)	119.9(120.3)	126.8(126.7)	121.2
N1-C6-H12	115.3(115.1)	116.7(116.4)	115.5(115.6)	----------
C5-C4-N3	113.3(113.8)	125.6(125.4)	118.5(119.0)	114.1
C4-C5-H11	118.1(118.3)	121.7(121.4)	121.0(121.6)	----------
C2-N3-H9	115.5(115.6)	----------	113.5(113.9)	----------
O10-C4-N3	120.3(120.5)	117.9(118.5)	120.0(118.8)	120.5
C4-N3-C2	128.3(127.7)	119.9(119.9)	124.2(124.0)	126.4
C4-N3-H9	116.1(116.6)	----------	122.1(121.9)	----------
N3C2O8	124.4(123.5)	125.6(124.9)	117.5(117.7)	----------
C6-N1-H7	121.4(121.2)	121.3(121.8)	----------	----------
C5-C6-H12	122.7(122.6)	123.2(123.2)	117.7(117.6)	----------
C4-O10-H13	----------	105.8(108.2)	115.0(113.5)	----------

a) In DFT B3LYP/6-31G (d, p)

b) In HF/6-31G (d, p)

Table2. Optimized geometries of the tautomers of cytosine.

Geometry	C1	C2	C3	C4	Exp.
		Bond length (Å)			
C4-N9	1.281a(1.256)b	1.358(1.343)	1.359(1.346)	1.319(1.345)	1.324
C5-C4	1.458(1.463)	1.441(1.445)	1.441(1.403)	1.340(1.370)	1.433
N3-C4	1.413(1.398)	1.320(1.296)	1.343(1.325)	1.323(1.347)	1.339
C6-C5	1.345(1.324)	1.358(1.338)	1.383(1.370)	1.456(1.407)	1.357
C2-N3	1.386(1.370)	1.372(1.362)	1.337(1.321)	1.323(1.410)	1.358
H12-C5	1.080(1.070)	1.082(1.071)	1.083(1.072)	1.079(1.071)	----------
N1-C6	----------	----------	1.342(1.327)	1.317(1.293)	----------
O8-C2	1.218(1.196)	1.219(1.196)	1.346(1.323)	1.220(1.194)	1.237
H13-C6	1.083(1.072)	1.085(1.074)	1.088(1.076)	1.079(1.079)	----------
H7-N1	1.008(0.993)	1.010(0.994)	----------	----------	----------
N9-H11	1.023(1.003)	1.007(0.992)	1.006(0.991)	1.010(0.991)	----------
N9-H10	----------	----------	1.004(0.990)	1.010(0.996)	----------
N3-H	1.011(0.996)	----------	----------	----------	----------
		Bond Angle(D)			
N9-C4-C5	121.6(121.6)	118.9(118.4)	122.3(122.1)	119.7(124.7)	----------
C4-C5-C6	120.0(119.8)	116.0(115.5)	116.0(115.5)	119.1(115.8)	117.0
C6-C5-H12	122.0(122.3)	----------	122.0(122.0)	120.4(122.0)	----------
C5-C6-N1	121.0	119.9(120.4)	124.1(124.2)	118.4(127.0)	121.2
N1-C6-H13	115.0	----------	115.7(115.7)	120.7(115.0)	----------
C5-C4-N3	113.0(113.5)	----------	121.0(121.0)	120.6(117.0)	----------
C4-C5-H12	122.0(117.7)	122.3(122.1)	122.0(122.1)	120.4(122.0)	----------
N9-C4-N3	125.1(124.8)	116.9(117.6)	116.5(116.8)	119.6(117.6)	118.3
C4-N3-C2	127.9(127.3)	120.2(120.4)	116.2(116.7)	121.5(124.7)	120.5
N3-C2-O8	123.0(122.7)	125.7(125.2)	----------	119.5(117.0)	----------
C6-N1-H7	121.0	121.6(121.4)	----------	----------	----------
C5-C6-H13	123.0(122.8)	123.1(123.0)	120.0(120.0)	120.7(117.0)	----------
C4-N9-H10	----------	122.2(122.1)	121.6(121.5)	120.0(119.8)	----------
C4-N9-H11	116.6(112.9)	117.8(118.1)	118.6(118.8)	120.0(122.0)	----------

a) In DFT B3LYP/6-31G (d, p)

b) In HF/6-31G (d, p)

2) The Relative energies and the dipole moments of tautomers.

The stability energies of tautomers of uracil and cytosine and their total dipole moments are listed in Table 3. Comparing these values of the total dipole moment with those calculated to experimental results (ref.13) has collected in table 3.The results of dipole moments at B3LYP level are better optimized than HF level

For charged species, the dipole moment is derived with respect to their center of mass, because for non-neutral molecules the calculated dipole moment depends on the origin of the coordinated system.

The use of molecular geometries determined in a 6-31G* basis set by optimized energy evaluation in an extended basis has obvious computational advantages for the calculation of tautomer energies.

The tautomerization or relative energy, E_{rel}, of a given form is defined as a difference between its total energy and more stable tautomer (U_1 and C_2).

The energy of U_1 is equal to – 214.8258 and – 412.4818 Hartree in DFT and HF levels, respectively.

The energy of C2 is equal to – 394.9413 and – 392.6310 Hartree in DFT and HF levels, respectively.

Table 3. Stability Energy, E_{rel}, in $kJmol^{-1}$ and the total dipole moments in degree at B3LYP and HF levels.

Structure	E_{rel}	μ
U_1	——	4.2[a] (4.7)[b] (4.2)[c]
U_2	50.80[a] (56.90)[b]	4.6 (5.2)
U_3	101.77 (112.73)	5.8 (6.1)
C_1	12.64 (9.67)	2.3 (2.5)
C_2	3.22 (3.88)	6.3 (7.1) (6.0 – 6.5)[c]
C_3	87.24 (30.16)	4.8 (4.9)
C_4	——	8.2 (8.5)

a) B3LYP/6-31G (d, p)
b) HF/6-31G (d, p)
c) From microwave study [17]

The indirect evidence suggests that C2 form predominates by factors of about 800 and 10^4 - 10^5 over forms 4, 1, respectively [9].

In the gas phase, cytosine exists in the amino-oxo form (C_2) [9]. A recent IR study of cytosine isolated in a low-temperature matrix has resulted in spectral assignments in terms of the amino-oxo form (2) and amino – hydroxy form (3) [10]. We have shown that IR frequencies computed for the amino – oxo form (2) is consistent with such an assignment and with the corresponding experimental data.

The experimental relative energy of U_2 is equal to 79.5 $kJmol^{-1}$ [7, 11]. Investigations of the tautomers of uracil in the gas phase and in low-temperature argon matrices using UV and IR spectroscopy have shown that for the uracil, the dike to form (U_1) is the only detectable form [11]. In total review, the calculated energies for all tautomers of the uracil and cytosine are greater than reported values [7].

Thus it is clear that accurate data concerning the stability of the tautomeric forms are consistent with HF level. Note that very recent DFT calculations predict the dipole moments of U_1 and C_2 much better than HF calculations.

3) Vibrational frequencies of IR spectra.

The calculated vibrational frequencies are necessary to make zero-point energy corrections. It is customary to scale the calculated harmonic frequencies in order to improve agreement with experiment, since it is well-known that the calculations consistently overestimate the vibrational frequencies.

Tables 4- 5 show the calculated harmonic wave numbers by DFT and HF levels for the tautomers of uracil and cytosine which are compared with the recorded IR spectra of the compounds in argon and nitrogen matrixes when available at 25˚C. The infrared spectra of uracil and cytosine have been reported in several papers [12-16].

For a better comparison of the predicted harmonic wave numbers with the recorded wave numbers of fundamental modes, we scaled the predicted wave numbers by 0.91 and 0.89 in the DFT and HF levels, respectively (the calculated wave numbers were scaled down to correct for anharmonicity and also electronic correlation in the case of the HF calculations). The absolute intensities from the DFT calculations are quite different from the corresponding HF intensities. The intensities from the DFT calculations are lower by about 20% than the intensities from

HF results. As to the predicted intensities from the DFT and HF calculations, it seems that both types of calculations predict the relative intensities more or less correctly.

The scaled DFT and HF wave numbers for the bond positions below 2000 cm^{-1} agree with the experimental wave numbers with similar accuracy.

For some modes, there is a disagreement between the DFT and HF calculations with regard to the assignment of the vibrational modes.

Note, however, that from the experimental point of view it is difficult to draw a correct conclusion about the nature of the modes in this spectral region, so we are unable to determine which type of assignment (DFT or HF) is correct.

Table4. Optimized wave numbers (cm^{-1}) from the bending vibrations of various groups in the U_1 f 25˚C, 37˚C and 40˚C in HF level.

T˚C	ßC5 H, ßC6H , ßN3H (DFT)	ßC5H, ßC6H, ßN3H (HF)	ßN1H (DFT)	ßN1H (HF)	νN3H (DFT)	νN3H (HF)
25	1197.7747	1305.6180	1506.1352	1646.5256	3619.6630	3863.1287
37	1197.7747	1306.0526	1506.1352	1647.0859	3619.6630	3863.6182
40	1197.7747	1306.0526	1506.1352	1647.0859	3619.6630	3863.6182

Table5. Results of wave numbers of C2 tautomer with DFT and HF calculations at the temperatures of 25˚C, 37˚C and 40˚C.

T˚C	Wave number(cm^{-1}) C2-DFT	Wave number (cm^{-1})C2-HF
25	1819.1632	1982.1202
37	1819.2486	1982.1202
40	1819.2486	1982.1202

(a) ***Uracil.*** Table4 shows that the band observed in the high-frequency region (3000-3700 cm^{-1}) originate from the stretching vibrations of OH and NH groups (modes νN1H, νN3H and νOH). In the low frequency region (1800-1850cm^{-1}) most band are due to stretching vibrations of the carbonyl groups (modes ν C2O, ν C4O). The strong band in 1407 cm^{-1} (in DFT) originating from the "in-plane" bending vibrations of the NH groups (β NH).

(b) ***Cytosine.*** Table5 shows that the bands observed in the high-frequency region (3400-3700cm^{-1}) originate from the stretching vibrations of OH, NH_2, and NH groups. The DFT assignments of these bands are the same as those from the HF/6-31G (d, p) calculations and agree with the experimental assignments [12]. In the lower frequency region (900–1800cm^{-1}) most bands are due to stretching vibrations of the ring, mixed with CH bending and scissoring of the amino-group. The positions and intensities of these bands are well predicted by the present DFT (B3LYP) calculations, and their assignments are the same as those from the HF/6-3lG (d, p) calculations. In the lower frequency region (below 900cm^{-1}), there are bands due to "in-plane" and "out-of-plane" vibrations. Modes 27 and 28 of the C_3 tautomer originate from the coupled "in-plane" and "out-of-plane" bending vibrations of the NH_2 group. Modes 28 and 31 of the C_2 tautomer originate from the "out-of-plane" ring deformation of NH_2, τ (to NH2), (to=torsion). For the tautomer C_2, the strong bands below 250cm^{-1} originating from the inversion of the amino group are also well predicted by the present calculations ,τ(inv NH2) ,(inv= inversion).

The results of vibrational frequencies for the most stable forms (U_1 and C_2) at 37˚C and 40˚C are compared with the results at 25˚C.

Mode 28 of the C_2 tautomer originate from the in-plane stretching vibration of the carbonyl group at the 37˚C is upper by about 0.03 cm^{-1} in HF level and about 0.08 cm^{-1} in DFT level with respect to corresponding data at the 25˚C.

In the HF level, the wave number in the high-frequency region originate from the bending vibrations of C_5H, C_6H and N_3H groups in the U_1 at the 37˚C is upper by about 0.56cm^{-1} with respect to corresponding data at the 25˚C (T1 = 25 ° C , T2= 37 ° C,T3 = 40 ° C)(Fig.3).

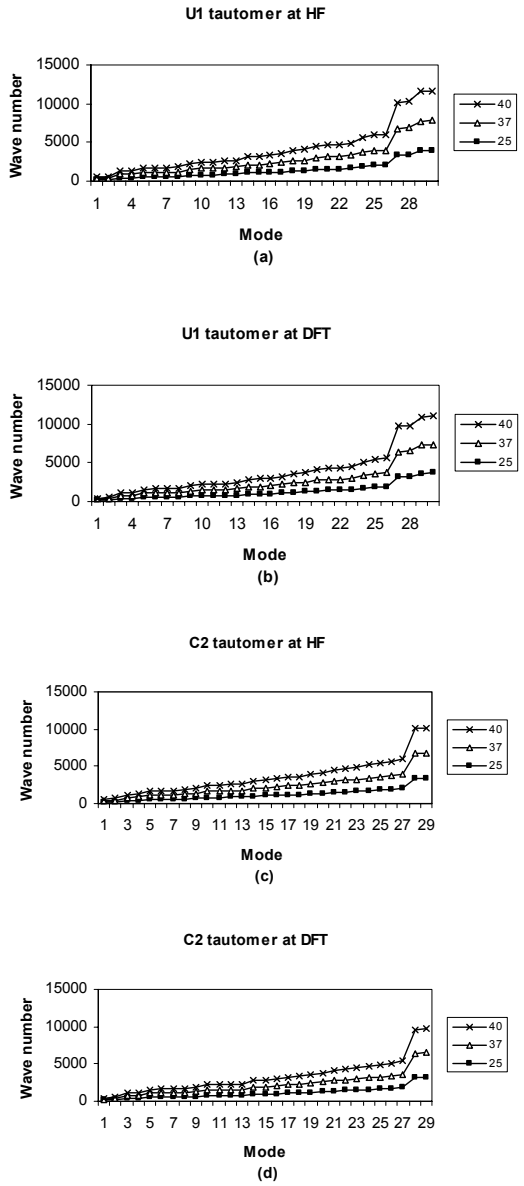


Fig.3. Curve of calculated IR wave numbers of U1tautomer of uracil with ab initio calculations at HF and DFT **(a), (b)** and C2 tautomer of cytosine **(c), (d)** at the different temperatures (25˚C, 37˚C and 40 ˚C) to vibrational modes.

CONCLUSIONS

The degree of agreement between correlated *ab initio* data and experimental methods mentioned will give us important insights into the nature of molecular interactions in the studied compounds and will provide us with an evaluation of the accuracy limits of these methods.

The major conclusions of the present theoretical study are the following:

Our results point out the possibility that the charge transfer electronic states may play a significant role in the, up to now, quite mysterious process of methods. It would be quite interesting to carry out a detailed experimental exploration of these systems using various techniques.

1) Optimization at the 6-31G* level yields molecular geometries in good agreement with experimental values for uracil and cytosine, and superior to those previously obtained theoretically.

2) The agreement between the theoretical bond length predicted in DFT level and the experimental geometry is very good for the ring atoms in U_1 and C_2 tautomers. The bond angles predicted by HF level give the better agreement with experiment.

3) The accurate data concerning the stability of the tautomeric forms are consistent with HF level, and the DFT calculations predict the dipole moments much better than HF calculations.

4) The scaled harmonic wave numbers of IR spectra of the cytosine and uracil tautomers predicted at the

DFT (B3LYP) /6-31G* level agree well with the recorded values for the normal modes at the 25˚C, and they are similar to the scaled harmonic wave numbers from the HF/6-31G* calculations.

5)By an accurate comparison of the wave numbers at the 25˚C, 37˚C and 40 ˚C, it seems that the significant changes occur in the bending vibration of N_3H group in the U_1 at the 37˚C in the HF level.

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