

THE STRUCTURAL, SPECTROSCOPIC, ELECTRONIC AND OPTICAL PROPERTIES OF THYMINE: A THEORETICAL INVESTIGATION

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Abstract

The structural, spectroscopic (IR, NMR and UV-Vis), electronic and optical properties of thymine (5-methylpyrimidine, C₅H₆N₂O₂) are investigated theoretically using DFT/B3LYP/6-311+G(d,p) level of theory. The calculated results are compared with available experimental data. The investigation of ¹H NMR chemical shift spectra of thymine shows that the maximum deviation occurs for highly deshielded protons compared to the experimental data. The calculated vibrational spectra analysis shows five distinct IR active mode of vibrations which are assigned as C=O stretching vibration, C=C stretching vibration, -CH₃ stretching vibration, free N-H vibration and free N-H vibration. It is noticeable that calculated IR spectrum of thymine have well agreement with the experimental results. The electronic and optical properties are calculated by time dependent-density functional theory (TD-DFT). A good agreement is obtained for the calculated optical absorption energy with the experimental value.

Keywords: Thymine, TD-DFT, IR, NMR, Electronic properties, Optical absorption

Introduction

Thymine is one of the four nucleic acid bases (adenine, guanine, cytosine and thymine) that constitutes the building blocks of life. Thymine encodes the genetic information of all organisms in deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) (Szyc et al. 2010; Samoylova et al. 2005; Canuel et al. 2005). It helps stabilize the nucleic acid portion of these molecules. These nucleobases absorb UV light strongly and act as primary chromophores of DNA (Pecourt et al. 2001; Kang et al. 2002; Gustavsson

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et al. 2010). Photo stability of DNA bases is the essential criteria for the preservation of genetic information of DNA bases. As nucleobases absorb UV light strongly, the knowledge of the electronic properties and excited state lifetimes are of paramount importance for understanding the UV radiation induced DNA damage (Ullrich et al. 2004; Ho et al. 2011; Kwok et al. 2008; Lange et al. 2009). Numerous experimental and theoretical studies on DNA and DNA bases have been performed to understand structural, electronic properties and excited state lifetimes (Middleton et al. 2009; Ghane et al. 2012; Varsano et al. 2006; Sobolewski et al. 2002; Alauddin et al. 2016). All the calculations have been focused on the ground state, higher electronic singlet and triplet states of the neutral molecules.

In this work, we have performed a theoretical investigation of structural, spectroscopic, electronic and optical properties of thymine with DFT/B3LYP level of theory and compared with the available experimental data. The optical properties have been calculated at the excited states using TD-DFT method.

Computational details

All the calculations have been performed using the hybrid approach of B3LYP as implemented in the quantum chemistry package G09 (Frisch et al. 2009). We have used 6-311+G (d,p) basis set in order to calculate the structure, IR, ¹H NMR and UV-Vis spectra of thymine in the ground state at the B3LYP level of theory. The optimized structure of thymine in the ground state was verified by calculating vibrational frequencies. There are several methods to calculate singlet excited states such as symmetry adapted cluster method/configuration interaction (SAC/CI) method (Honda et al. 2002), configuration interaction singles (CIS) method (Drougas et al. 2006) and time dependent density functional theory (TD-DFT) (Varsano et al. 2006) calculations. Among them, the TD-DFT is new approach for studying electronic excitations which gives more accurate results (Varsano et al. 2006). Therefore, excited state calculations have been done by TD-DFT method employing B3LYP function and 6-311+G(d,P) basis set to study electronic and optical properties of thymine. To avoid complexity, we have calculated only six lowest excited states of thymine.

Results and Discussion

Molecular geometry and thermodynamic parameters

The optimized structure of thymine by DFT/ B3LYP is shown in Fig. 1. For optimizing minimum energy structure of thymine, first we have done intrinsic reaction coordinate (IRC) calculation with HF/6-31G. This minimum energy structure was re-optimized using DFT/B3LYP/6-311+G (d,p) level of theory. The final geometry corresponds to true energy minima as revealed by the lack of imaginary frequencies in the vibrational mode calculation. The optimized bond distances and bond angles are compared with available experimental data (Clowney et al. 1996) in Table 1. It is observed that the bond distances and bond angles have been converged within very short deviation with respect to the experimental values.

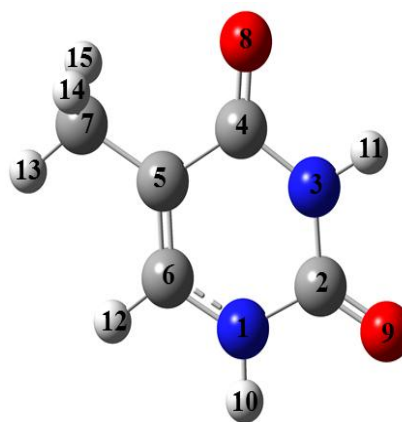


Fig. 1. Optimized structure of thymine

Due to the presence of electron withdrawing atoms (O) in thymine, electron density is not delocalized on the aromatic ring. On the contrary, methyl group helps to partially delocalize electron density (formation of double bond between C5 and C6) as it acts as an electron donating group.

Table 1. Comparison of calculated bond lengths (Å) and bond angles (°) of thymine with available experimental data.

	Calc.	Expt.
Bond Length (Å)		
N(1)-C(2)	1.387	1.376
C(2)-N(3)	1.384	1.373
N(3)-C(4)	1.381	1.381
C(4)-C(5)	1.467	1.446
C(5)-C(6)	1.349	1.339
C(6)-N(1)	1.380	1.379
Bond Angle (°)		
N(1)-C(2)-N(3)	112.5	114.4
C(2)-N(3)-C(4)	128.0	127.1
N(3)-C(4)-C(5)	114.6	115.3
C(4)-C(5)-C(6)	118.0	118.0
C(5)-C(6)-N(1)	122.8	123.6
C(6)-N(1)-C(2)	123.8	121.2

Thermo dynamical properties such as energy, specific heat capacity, entropy, enthalpy and dipole moment were studied theoretically. The total energy (SCF), zero point vibrational energy (ZPVE), rotational constants, specific heat capacity (C_v) at constant volume, entropy (S) and dipole moment (μ) of thymine are provided in the Table 2.

Table 2. Calculated thermodynamic parameters of thymine.

Molecule	SCF energy/ Hartree	Z PV energy/ kcal mole ⁻¹	Rotational constant /GHz	Specific heat(C_v)/cal mol ⁻¹ K ⁻¹	Entropy (S)/cal mol ⁻¹ K ⁻¹	Dipole moment, μ /Debye
Thymine	-454.2756	71.6912	3.19681 1.39821 0.97859	29.443	87.173	4.5318

SCF = Self-Consistent Field and ZPV = Zero Point Vibrational

Thermodynamics parameters can be changed depending upon the level of computational theory. To get more accurate results very high level computational calculations such as Møller-Plesset perturbation theory (MP2) is required.

¹H NMR spectral analysis

The NMR spectroscopy is one of the most powerful techniques for the structural analysis of organic compounds. It is well-established that the combined use of experimental NMR spectroscopic technique and computational simulation methods give a powerful gadget to interpret and predict the structure of organic compound, even for the structures of large biomolecules. To get theoretical ¹H NMR results of thymine, first the full geometry optimization is carried out with the B3LYP/6-311G+(d, p) in gas phase. After optimization, ¹H NMR calculations of the studied compounds is calculated by using the gauge-including atomic orbital (GIAO) method. The chemical shifts of the studied compounds are reported in ppm relative to tetramethylsilane (TMS) for ¹H NMR spectrums presented in Table 3.

Table 3. Calculated ^1H NMR data of thymine.

Molecule	Protons with number	Chemical Shift (Calc.)/ppm	Chemical Shift (Expt.)/ppm
Thymine	H(11)	6.88	11.0
	H(12)	6.64	10.6
	H(10)	5.92	7.27
	H(14)	1.97	1.75
	H(15)	1.97	1.75
	H(13)	1.25	1.75

H(11), H(12) and H(10) protons are highly deshielded due to the attachment of two oxygen atoms with neighboring carbon atoms compared to the methyl protons. Therefore, the chemical shifts move to the higher frequency region. On the other hand, the chemical shifts move to the lower frequency region for methyl protons H(13), H(14) and H(15) as these are all shielded. The experimental ^1H NMR spectra of thymine in DMSO solvent are shown in Table 3 (www.chemicalbook.com). The difference of chemical shifts between theoretical and experimental results is larger in the higher frequency regions due to the solvent effect. DMSO is a very useful solvent for NMR spectroscopy due to its ability to dissolve a wide range of analytes, the simplicity of its own spectrum and its suitability for high-temperature NMR spectroscopic studies. However, it has some disadvantages such as high viscosity which broadens signals and hygroscopicity which leads to an overwhelming H_2O resonance in the ^1H NMR spectrum. In contrast, all twelve hydrogen atoms in TMS are equivalent, its ^1H NMR spectrum consists of a singlet. The chemical shift of this singlet is assigned as $\delta 0$ and all other chemical shifts are determined relative to it. It is reported that chemical shift varies from 0.3 to 4.6 ppm due to the hydrogen bonding (Abraham et al. 2006). Hydrogen bonding makes the difference in chemical shifts between TMS in CDCl_3 and DMSO solvent. On the other hand, a reasonable agreement is found between theoretical and experimental results in the lower frequency region.

Vibrational spectral analysis

The assignments of the vibrational frequencies are shown in Table 4. In the calculated spectrum of thymine, five IR active mode of vibrations were found at 1682, 1726, 2913, 3452 and 3494 cm^{-1} which are assigned as C2=O stretching vibration, C4=O stretching vibration, -CH₃ stretching vibration, free N3-H vibration and free N1-H vibration respectively. Five IR absorption bands at 1720, 1770, 2950, 3435 and 3481 cm^{-1} were observed experimentally which are assigned as C2=O stretching vibration, C4=O stretching vibration, -CH₃ stretching vibration, free N3-H vibration and free N1-H vibration respectively (Plutzer et al. 2003). Calculated IR spectrum of thymine have well agreement with the experimental results.

Table 4. IR calculated and experimental frequencies of thymine.

Molecule	IR calculated frequencies/ cm^{-1}	IR experimental frequencies/ cm^{-1}	IR active mode of vibration
Thymine	1682	1720	C2=O stretching vibration
	1726	1770	C4=O stretching vibration
	2913	2950	-CH ₃ stretching vibration
	3452	3435	Free N3-H vibration
	3494	3481	Free N1-H vibration

Calculated values were corrected by multiplying the frequency factor, $f = 0.960$

Electronic absorption and optical properties

We have used TD-DFT method for the calculations of excited state properties of thymine for excitation from the ground state to the higher electronic states. For this purpose, we have calculated six lowest singlet electronic states from the ground state of thymine. The computed electronic values such as absorption wavelength (λ), excitation energies (E) and oscillator strengths (f) are tabulated in Table 5. The major transition with highest oscillator strength for thymine molecule is at 248 nm (5.00 eV).

Table 5. Calculated wavelengths λ (nm), excitation energies E(eV) and oscillator strength (f) and major electronic transitions of thymine.

Molecule	λ (nm)	E (eV)	f	Major transitions
Thymine	249	4.98	0.1373	H \rightarrow L (93%), H-2 \rightarrow L (2%) & H-2 \rightarrow L+2 (3%)
	207	5.99	0.0695	H-2 \rightarrow L (87%) & H \rightarrow L+2 (7%)

H = HOMO & L = LUMO

This transition occurs from HOMO to LUMO (93%) ($\pi \rightarrow \pi^*$) with two minor transitions from HOMO-2 to LUMO (2%) and HOMO-2 to LUMO+2 (3%). The second major transition occurs at 207 nm (5.99 eV) from HOMO-2 to LUMO (87%) and HOMO to LUMO+2 (7%). The HOMO and LUMO that participate in the lowest electronic transitions of thymine are presented in Fig. 2 as a Jablonski diagram.

Theoretical and experimental UV-Visible absorption spectrum of thymine are shown in Fig.3. From the figure it is very clear that thymine is a colorless compound as it does not absorb any visible light. The experimental UV-Visible absorption spectrum of thymine (*PhotochemCad*) is at 264 nm which shows that our calculated spectrum is in very good agreement with experiment. Little red shift from 249 nm to 264 nm is due to the solvent used to measure UV-Visible absorption spectrum of thymine.

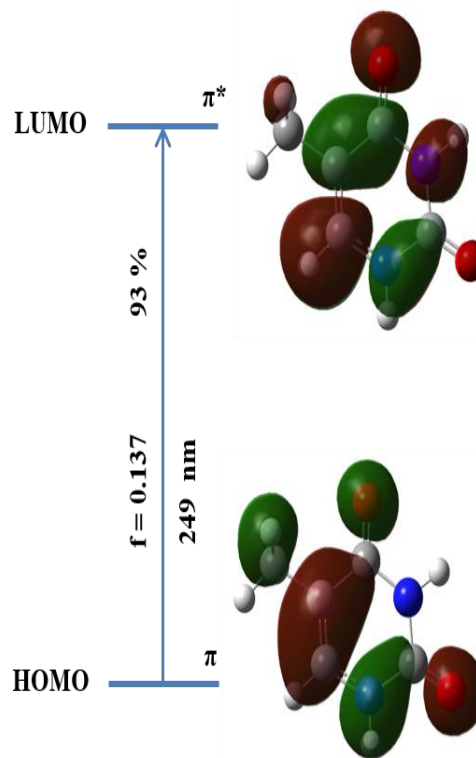


Fig. 2. Jablonski diagram and singlet excited-state transition from HOMO \rightarrow LUMO of thymine.

The frontier molecular orbital's (FMO) play an important role in the optical and electronic properties. In the present study, the FMO energy gap is calculated as the energy difference between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) as follows:

$$\text{HOMO}_{\text{energy}} (\text{B3LYP}) = -6.99\text{eV},$$

$$\text{LUMO}_{\text{energy}} (\text{B3LYP}) = -1.57\text{eV}$$

$$\text{HOMO-LUMOenergy gap (B3LYP)} = 5.42\text{eV}$$

Therefore, the energy gap of FMO of thymine is 5.42 eV.

Conclusion

The molecular geometry optimization, thermodynamic properties, vibration frequency assignments, electronic and optical properties of thymine molecule were studied theoretically. The calculated ^1H NMR chemical shift spectra are not in well accord with the experimental data due to the solvent used for measurement. This discrepancy occurred due to the solvent. The calculated IR active mode of vibrations and their assignments are in well agreement with the available experimental data. The investigation of electronic properties shows that the HOMO-LUMO energy band gap of thymine at B3LYP level is 5.42 eV. The calculation of optical properties at excited states shows that the major transition (from HOMO to LUMO (93%) ($\pi \rightarrow \pi^*$) occur sat 249 nm (4.98 eV) with a minor transition at 207 nm (5.99 eV). Our theoretical UV-Visible absorption spectrum suggests that thymine is a white compound as it does not absorb any visible light which is in good agreement with experiment.

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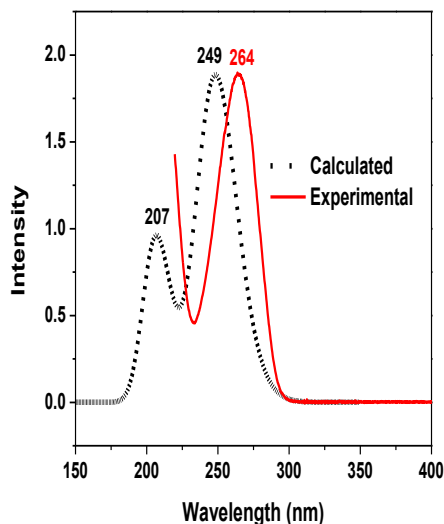


Fig. 3. Calculated and experimental UV-Visible absorption spectrum of thymine

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