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COMPARATIVE STRUCTURAL AND VIBRATIONAL STUDY ON THE MOLECULAR STRUCTURE OF THREONINE AND TYROSINE BY AB INITO AND DENSITY FUNCTIONAL THEORY

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ABSTRACT
A brief structural and vibrational study has been performed on the molecular structure of two well known amino acids threonine and tyrosine. The equilibrium geometry, harmonic vibrational frequencies, and infrared intensities were calculated by the ab initio HF and density functional B3LYP method employing 6-311++G(d,p) and Aug-cc-pVDZ as the basis sets. A detailed interpretation of the infrared spectra of threonine and tyrosine is reported in the present work. The similarities and differences between the vibrational spectra of the two molecules studied have been highlighted. The calculations are in agreement with experiment. The theromodynamic calculations related to the title compounds were also performed at HF and B3LYP levels of theory employing 6-311++G(d,p) and Aug-cc-pVDZ basis sets respectively. The FTIR spectra of tyrosine were recorded in solid and liquid phases respectively.

KEYWORDS: FT-IR spectra, Ab initio and DFT calculations, Threonine and Tyrosine, Vibrational study.

1. INTRODUCTION
Threonine (2-amino-3-hydroxy-butanecarboxylic acid) is a member of aliphatic hydroxy amino acid. It is an important amino acid found in several proteins of human beings such as γ-globulin, β-lactoglobulin, hemoglobin, insulin, silk fibroin and among others.[1] Investigation of threonine is relevant for the physical point of view of both owing to the possibility to observe the behaviour of a system where the hydrogen bond plays a fundamental role[2-4] and the technological importance of a material which shows second harmonic conversion efficiency greater than 1 relative to potassium dihydrogen phosphate.[5] Requirements for Threonine appear to decrease with age and increase with stress. It is necessary for the formation of tooth enamel, elastin and collagen which are needed for both healthy skin and wound healing. Threonine also plays a role in preventing fat from accumulating in the liver. A fatty liver can affect liver function and is associated with diseases of the liver such as cirrhosis. Threonine has been investigated extensively by various authors.[6-11] Infrared (IR) absorption measurements and polarized Raman Scattering results for L-threonine at room temperature and assignments of some internal modes are also reported by Freire et al.[12] Schäfer and coworkers[7] considered gaseous threonine on the basis of favorable intramolecular interaction and yielded possible conformer. The ionization energies of four conformer of threonine has been calculated by Powis et al[8] and compared with their synchrotron radiation photoelectron spectroscopy experiment. The orbital dependent vertical ionization energies of four threonine conformer have been studied by Dehareng et al[9] using the outer valence green's function (OGVF) technique.

Tyrosine is a nonessential amino acid that is synthesized in the body from phenylalanine. As a building block for several important brain chemicals, tyrosine is needed to make epinephrine, norepinephrine, serotonin, and dopamine, all of which work to regulate mood. Deficiencies in tyrosine, therefore, have been associated with depression. Tyrosine also aids in the production of melanin (pigment responsible for hair and skin color) the dark pigment which protects against the harmful effects of ultraviolet. Therefore tyrosine may be valuable aid in treating vitiligo, and in the function of organs in the body responsible for making and regulating hormones, including the adrenal, thryroid, and pituitary glands. Tyrosine is also involved in the synthesis of enkephalins, substances that have pain-relieving effects in the body. Low levels of tyrosine have been associated with low blood pressure, low body temperature, and an under active thyroid. Because tyrosine binds unstable molecules (called free radicals) that can potentially cause

damage to the cells and tissues, it is considered as a mild antioxidant. Thus, tyrosine may be useful for people who have been exposed to harmful chemicals (such as from smoking) and radiation. Tyrosine in gas phase has been well studied.[13, 14] Earlier, Hameka et al[15] has been reported the ground state (S_0) energy at HF/6-31G level and first singlet excited state (S_1) energy at CIS/6-31G level of theory for tyrosine and also interpreted the absorption and fluorescence spectra reported previously by Teale and Weber.[16] Recently, the structure and conformational behaviour of neutral tyrosine using the experimental technique of matrix-isolation high resolution Fourier Transform Infrared Spectroscopy in combination with DFT/B3LYP/6-31++G(d,p) computational quantum chemistry has been carried out by Ramaekers et al.[17] The experimental result of the ultraviolet excitation and fluorescence spectra of tyrosine have been reported earlier.[18-20] The work of S. Hill has indicated that the tyrosine molecule may also exhibit phosphorescence under certain conditions.[21] Samuels et al.[22] reported computational results at the CASSCF(6,6)/6-31G level of theory for two lowest singlet state S_0 and S_1 and for the two lowest triplet states T_1 and T_2 of the tyrosine molecule where S_0 being the molecular ground state.

Recently, the three hydroxy amino acids (serine, threonine and tyrosine) have been studied at the Hartree-Fock level of theory based on geometry optimization for one initial structure for each molecule by B. Lakard.[10] We can observe that the amino acids (H_2N-CHR-COOH) studied here only differ by their –R group, which contain a secondary alcohol for threonine and a primary aromatic alcohol for tyrosine. Therefore, it can be interesting to compare the physiochemical properties common to these two amino groups. The purpose of this investigation to study the molecular structure, total electronic energy, dipole moments, polarizabilities, rotational constants and vibrational spectra of the two most important hydroxy amino acids (threonine and tyrosine). Furthermore, the general assignments of their fundamental frequencies are proposed by ab initio DFT calculation and compared with observed IR spectrum. In present study, electron correlation has been included using the DFT approximations with different basis sets. Density functional theory calculations are already reported to provide excellent agreement with experimental vibrational frequencies for organic compounds, if the calculated frequencies are scaled appropriately.[23-29]

2. METHODOLOGY

2.1 Experimental study

Hydroxyamino acids (threonine and tyrosine) samples used in the present investigation was obtained from Merck (99% purity) have been used as such without further purification to record the infrared (IR) spectrum in a nujoll mull using a Fourier Transform IR spectrometer (JASCFTIR-5300) with a resolution of 2 cm^{-1} in the range of 400-4000 cm^{-1}. The IR spectrum of the molecules in a K Br pellet was also recorded on a Perkin Elmer RX1, FT-IR spectrometer in the range 450-4000 cm^{-1} with a resolution of 1 cm^{-1}.

2.2 Computational methodology

The geometry optimizations and frequency calculations have been carried out with the Hartree-Fock[30] and density functional theory (DFT). Here, we have used the hybrid of Becke's non-local three parameter exchange and correlated functional with the Lee- Yang-Parr correlation functional (B3LYP) methods[31] using different basis sets viz; 6-311++G (d, p) and Aug-cc-pVDZ for the molecular orbital expansion without imposing any symmetry constraints. The optimized geometries at the B3LYP/6-311++G(d,p) level are used as starting geometry for optimization at the DFT/B3LYP/Aug-cc-pVDZ level of theory. The frequency calculations were carried out at same level as the respective optimization processes using analytic evaluation of the second derivatives of energy with respect to the nuclear displacement. The DFT/B3LYP method has been widely applied to obtain the conformational behaviour, theoretical vibrational frequencies and infrared intensities of amino acids, which are well agreed with the experimental data for alanine[32], glycine[33], Proline[34], tyrosine[35] and valine.[36] All calculations in the present work were carried out using Gaussian 09[37] suite of ab initio quantum mechanical program.

3. RESULTS AND DISCUSSION

3.1 Geometry and relative energies

The optimized geometrical parameter (i.e. bond lengths, bond angles and dihedral angles) of hydroxyl amino acids (threonine and tyrosine) at RHF and DFT/B3LYP level of theories by employing 6-311++G(d,p) and Aug-cc-pVDZ basis sets in gas phase are listed in Tables 1 and 2 respectively. The experimental values reported earlier by Samuels et al[22] for the tyrosine are also included in Table 2. The molecular structures of threonine and tyrosine are shown in Figures 1 and 2 respectively.

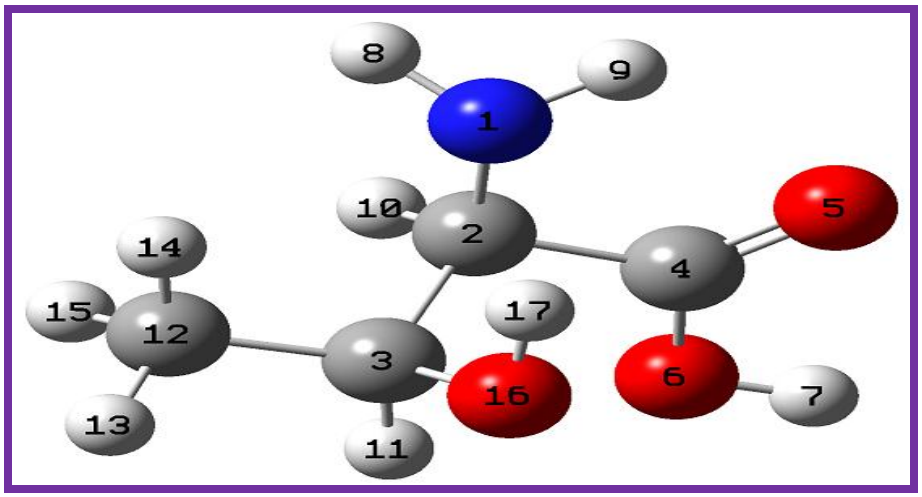

Fig 1: Molecular structure of threonine molecule obtained at the DFT/B3LYP/Aug-cc-pVDZ level of theory.

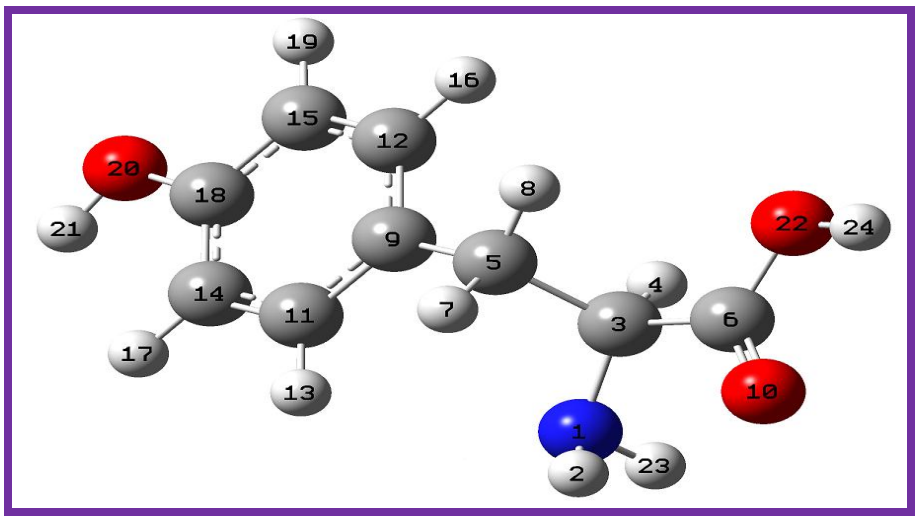

Fig 2: Molecular structure of tyrosine molecule obtained at the DFT/B3LYP/Aug-cc-pVDZ level of theory.

Table 1: Geometrical parameters of threonine using RHF and B3LYP methods by employing 6-311++G(d,p) and Aug-cc-pVDZ basis sets respectively.

Geometrical parameters[a]	RHF		B3LYP	
	6-311++G(d, p)	Aug-cc-pVDZ	6-311++G(d, p)	Aug-cc-pVDZ
Bond lengths (Å)				
N1-C2	1.451	1.453	1.463	1.464
N1-H8	0.997	0.999	1.011	1.014
N1-H9	0.999	1.001	1.015	1.017
C2-C3	1.541	1.540	1.556	1.555
C2-C4	1.518	1.518	1.523	1.522
C2-H10	1.090	1.095	1.099	1.104
C3-H11	1.082	1.086	1.091	1.097
C3-C12	1.524	1.524	1.529	1.529
C3-O16	1.399	1.403	1.420	1.422
C4-O5	1.183	1.189	1.206	1.212
C4-O6	1.324	1.328	1.350	1.353
O6-H7	0.946	0.948	0.969	0.971
C12-H13	1.084	1.089	1.092	1.097
C12-H14	1.086	1.090	1.094	1.099
C12-H15	1.087	1.092	1.095	1.100
O16-H17	0.945	0.946	0.970	0.972
Bond angles (°)				
C2-N1-H8	112.2	111.9	112.4	112.2

C2-N1-H9	111.4	110.9	110.5	110.1
H8-N1-H9	109.1	108.8	109.0	108.7
N1-C2-C3	110.2	110.3	109.4	109.5
N1-C2-C4	109.3	109.2	109.2	109.2
N1-C2-H10	112.8	112.9	112.9	112.9
C3-C2-C4	110.9	110.9	111.3	111.3
C3-C2-H10	108.3	108.2	108.2	108.0
C4-C2-H10	105.3	105.2	105.9	105.8
C2-C3-H11	108.6	108.5	108.8	108.8
C2-C3-C12	112.0	112.0	111.6	111.6
C2-C3-O16	109.5	109.5	108.9	108.9
H11-C3-C12	108.9	108.9	109.5	109.5
H11-C3-O16	106.1	106.0	106.2	106.0
C12-C3-O16	111.6	111.6	111.7	111.7
C2-C4-O5	124.9	124.9	125.0	125.0
C2-C4-O6	112.3	112.5	112.1	112.2
O5-C4-O6	122.7	122.6	122.9	122.8
C4-O6-H7	108.9	108.4	107.4	107.0
C3-C12-H13	109.4	109.4	109.5	109.5
C3-C12-H14	111.3	111.2	111.0	111.0
C3-C12-H15	111.1	110.9	111.2	111.0
H13-C12-H14	108.0	108.1	107.9	108.1
H13-C12-H15	108.3	108.4	108.4	108.4
H14-C12-H15	108.7	108.7	108.7	108.8
C3-O16-H17	108.2	108.1	105.6	105.6
Dihedral angles (°)				
H8-N1-C2-C3	-95.9	-94.6	-101.7	-101.5
H8-N1-C2-C4	142.0	143.2	136.3	136.4
H8-N1-C2-H10	25.2	26.6	18.8	18.9
H9-N1-C2-C3	141.5	143.7	136.3	137.3
H9-N1-C2-C4	19.4	21.5	14.3	15.2
H9-N1-C2-H10	-97.4	-95.1	-103.2	-102.3
N1-C2-C3-H11	-174.0	-174.8	-168.3	-168.8
N1-C2-C3-C12	65.6	64.9	70.8	70.2
N1-C2-C3-O16	-58.6	-59.5	-53.0	-53.6
C4-C2-C3-H11	-52.9	-53.6	-47.6	-47.9
C4-C2-C3-C12	-173.2	-173.9	-168.5	-168.9
C4-C2-C3-O16	62.5	61.7	67.7	67.3
H10-C2-C3-H11	62.2	61.3	68.3	67.9
H10-C2-C3-C12	-58.1	-59.0	-52.6	-53.1
H10-C2-C3-O16	177.6	176.6	-176.4	-176.9
N1-C2-C4-O5	-5.4	-6.19	-4.7	-5.1
N1-C2-C4-O6	176.9	176.3	176.8	176.5
C3-C2-C4-O5	-127.1	-127.9	-125.5	-126.1
C3-C2-C4-O6	55.2	54.5	55.9	55.5
H10-C2-C4-O5	116.0	115.2	117.2	116.8
H10-C2-C4-O6	-61.7	-62.3	-61.4	-61.6
C2-C3-C12-H13	179.7	179.3	-177.9	-178.3
C2-C3-C12-H14	-61.0	-61.4	-58.8	-59.1
C2-C3-C12-C15	60.2	59.8	62.4	61.9
H11-C3-C12-H13	59.6	59.2	61.6	61.1
H11-C3-C12-H14	178.8	178.6	-179.3	-179.7
H11-C3-C12-H15	-59.9	-60.3	-58.1	-58.6
O16-C3-C12-H13	-57.2	-57.4	-55.8	-56.1
O16-C3-C12-H14	62.1	61.9	63.3	63.1
O16-C3-C12-H15	-176.7	-176.9	-175.5	-175.8
C2-C3-O16-H17	51.9	51.7	46.8	46.6
H11-C3-O16-H17	168.9	168.6	163.8	163.6

C12-C3-O16-H17	-72.5	-72.9	-76.9	-77.1
C2-C4-O6-H7	-179.8	179.7	-179.5	179.9
O5-C4-O6-H7	2.4	2.1	1.9	1.5

[a] See Fig. 1 for the atom numbering.

Table 2: Geometrical parameters of tyrosine using RHF and B3LYP methods by employing 6-311++G(d, p) and Aug-cc-pVDZ basis sets respectively.

Geometrical parameters[a]	RHF		B3LYP		Ref.[b]
	6-311++G(d, p)	Aug-cc-pVDZ	6-311++G(d, p)	Aug-cc-pVDZ	
Bond lengths (Å)					
N1-H2	1.001	1.004	1.017	1.019	1.040
N1-C3	1.443	1.445	1.455	1.457	1.503
N1-H23	0.999	1.002	1.014	1.017	1.045
C3-H4	1.084	1.088	1.093	1.098	-
C3-C5	1.546	1.546	1.557	1.557	1.540
C3-C6	1.523	1.523	1.529	1.529	1.526
C5-H7	1.087	1.091	1.095	1.100	-
C5-H8	1.084	1.089	1.093	1.098	-
C5-C9	1.513	1.514	1.511	1.512	1.507
C6-O10	1.184	1.189	1.206	1.212	1.217
C6-O22	1.329	1.333	1.355	1.359	1.332
C9-C11	1.387	1.389	1.398	1.402	1.409
C9-C12	1.391	1.394	1.400	1.404	1.411
C11-H13	1.075	1.079	1.083	1.089	-
C11-C14	1.386	1.389	1.393	1.397	1.402
C12-C15	1.382	1.386	1.391	1.396	1.385
C12-H16	1.077	1.082	1.086	1.092	-
C14-H17	1.077	1.082	1.086	1.092	-
C14-C18	1.384	1.388	1.395	1.399	1.402
C15-C18	1.385	1.388	1.394	1.399	1.406
C15-H19	1.074	1.079	1.083	1.089	-
C18-O20	1.352	1.356	1.371	1.374	1.359
O20-H21	0.941	0.942	0.963	0.965	0.974
O22-H24	0.946	0.948	0.969	0.972	0.984
Bond angles (°)					
H2-N1-C3	110.5	110.1	109.7	109.4	-
H2-N1-H23	107.0	106.6	106.7	106.2	-
C3-N1-H23	111.4	111.0	110.9	110.7	-
N1-C3-H4	108.9	108.9	108.9	108.9	-
N1-C3-C5	110.9	110.9	110.8	110.7	-
N1-C3-C6	112.7	112.7	112.9	112.9	-
H4-C3-C5	108.3	108.3	107.9	108.0	-
H4-C3-C6	107.0	107.1	107.6	107.7	-
C5-C3-C6	108.7	108.6	108.6	108.4	-
C3-C5-H7	108.0	107.9	107.6	107.5	-
C3-C5-H8	109.0	108.9	108.7	108.7	-
C3-C5-C9	113.6	113.5	113.5	113.3	-
H7-C5-H8	106.9	107.0	107.0	107.2	-
H7-C5-C9	110.2	110.2	110.7	110.6	-
H8-C5-C9	108.9	109.0	109.2	109.4	-
C3-C6-O10	125.2	125.2	125.3	125.3	-
C3-C6-O22	112.3	112.5	112.1	112.2	-
O10-C6-O22	122.4	122.3	122.6	122.5	-
C5-C9-C11	121.9	121.9	121.8	121.7	-
C5-C9-C12	120.4	120.5	120.4	120.5	-
C11-C9-C12	117.6	117.6	117.8	117.8	-
C9-C11-H13	119.7	119.8	119.5	119.5	-
C9-C11-C14	121.3	121.4	121.2	121.3	-

H13-C11-C14	121.3	118.9	119.3	119.2	-
C9-C12-C15	121.8	121.8	121.7	121.7	-
C9-C12-H16	119.5	119.5	119.5	121.7	-
C15-C12-H16	118.7	118.6	118.8	118.8	-
C11-C14-H17	119.9	119.9	119.9	120.0	-
C11-C14-C18	119.9	119.9	120.0	119.9	-
H17-C14-C18	120.2	120.2	120.0	120.1	-
C12-C15-C18	119.6	119.5	119.5	119.5	-
C12-C15-H19	121.2	121.2	121.3	121.3	-
C18-C15-H19	119.3	119.3	119.2	119.3	-
C14-C18-C15	119.7	119.8	119.8	119.9	-
C14-C18-O20	122.6	122.6	122.7	122.7	-
C15-C18-O20	117.7	117.6	117.5	117.4	-
C18-O20-H21	110.9	110.9	109.6	109.5	-
C6-O22-H24	108.9	108.6	107.5	107.1	-
Dihedral angles (°)					
H2-N1-C3-H4	164.6	164.2	161.7	161.6	-
H2-N1-C3-C5	-76.3	-76.6	-79.8	-79.8	-
H2-N1-C3-C6	45.9	45.5	42.2	42.0	-
H23-N1-C3-H4	45.8	46.4	44.0	44.9	-
H23-N1-C3-C5	164.9	165.6	162.5	163.5	-
H23-N1-C3-C6	-72.9	-72.3	-75.4	-74.7	-
N1-C3-C5-H7	50.5	51.3	50.6	51.8	-
N1-C3-C5-H8	166.3	167.1	166.1	167.5	-
N1-C3-C5-C9	-72.1	-71.2	-72.2	-70.7	-
H4-C3-C5-H7	169.9	170.9	169.7	170.9	-
H4-C3-C5-H8	-74.2	-73.3	-74.7	-73.3	-
H4-C3-C5-C9	47.4	48.4	46.9	48.5	-
C6-C3-C5-H7	-74.0	-73.1	-73.9	-72.6	-
C6-C3-C5-H8	41.8	42.7	41.6	43.1	-
C6-C3-C5-C9	163.4	164.4	163.3	164.9	-
N1-C3-C6-O10	-19.5	-20.9	-22.1	-23.6	-
N1-C3-C6-O22	161.5	160.2	159.3	157.9	-
H4-C3-C6-O10	-139.3	-140.7	-142.3	-143.8	-
H4-C3-C6-O22	41.7	40.4	39.2	37.7	-
C5-C3-C6-O10	103.9	102.5	101.2	99.5	-
C5-C3-C6-O22	-75.0	-76.4	-77.4	-78.9	-
C3-C5-C9-C11	84.9	86.3	79.7	84.3	-
C3-C5-C9-C12	-94.8	-93.0	-99.5	-94.8	-
H7-C5-C9-C11	-36.5	-34.9	-41.3	-36.5	-
H7-C5-C9-C12	143.7	145.7	139.4	144.4	-
H8-C5-C9-C11	-153.4	-152.1	-158.8	-154.4	-
H8-C5-C9-C12	26.8	28.5	21.9	26.5	-
C3-C6-O22-H24	178.1	177.8	177.6	177.4	-
O10-C6-O22-H24	-0.9	-1.13	-1.1	-1.15	-
C5-C9-C11-H13	-0.2	0.16	-0.1	0.3	-
C5-C9-C11-C14	-179.7	-179.4	-179.2	-179.0	-
C12-C9-C11-H13	179.5	179.6	179.1	179.4	-
C12-C9-C11-C14	0.09	0.01	0.03	0.1	-
C5-C9-C12-C15	179.5	179.2	179.2	178.9	-
C5-C9-C12-H16	-0.72	-1.07	-1.16	-1.4	-
C11-C9-C12-C15	-0.25	-0.19	-0.08	-0.2	-
C11-C9-C12-H16	179.5	179.5	179.6	179.5	-
C9-C11-C14-H17	-179.9	-179.9	-179.9	-179.9	-
C9-C11-C14-C18	0.11	0.19	0.07	0.1	-
H13-C11-C14-H17	0.61	0.52	0.99	0.7	-
H13-C11-C14-C18	-179.3	-179.4	-179.0	-179.2	-
C9-C12-C15-C18	0.19	0.16	0.03	0.08	-

C9-C12-C15-H19	179.8	179.8	179.7	179.7	-
H16-C12-C15-C18	-179.6	-179.5	-179.6	-179.6	-
H16-C12-C15-H19	0.06	0.10	0.03	0.07	-
C11-C14-C18-C15	-0.17	-0.20	-0.12	-0.2	-
C11-C14-C18-O20	-179.9	-179.9	-179.9	-179.9	-
H17-C14-C18-C15	179.9	179.9	179.9	179.8	-
H17-C14-C18-O20	0.12	0.12	0.12	0.13	-
C12-C15-C18-C14	0.02	0.06	0.07	0.13	-
C12-C15-C18-O20	179.8	179.8	179.8	179.9	-
H19-C15-C18-C14	-179.6	-179.6	-179.6	-179.5	-
H19-C15-C18-O20	0.18	0.18	0.16	0.21	-
C14-C18-O20-H21	-0.09	-0.24	-0.001	-0.29	-
C15-C18-O20-H21	-179.9	179.9	-179.	179.9	-

[a] See Fig. 2 for the atom numbering. [b] Ref.[22]

In case of threonine, all the bond lengths increases, except the CC bond length as the basis set changed from 6-311++G(d,p) to Aug-cc-pVDZ at both level of calculations (See Table 1). The change in basis set does not change the CC bond lengths and it remains intact at both levels of theories. The bond angles of threonine found at the B3LYP/6-311++G(d,p) and B3LYP/aug-cc-pVDZ levels are slightly less than the corresponding bond angles found at the RHF/6-311++G(d,p) and RHF/aug-cc-pVDZ levels. Table 2 shows the geometrical parameter of tyrosine molecule. The changes in geometrical parameters of tyrosine follow similar trends like threonine.

The amino acids (H_2N-CHR-COOH) studied here only differ by their -R group, which contain a secondary alcohol for threonine and a primary aromatic alcohol for tyrosine. Here, we may compare the bond lengths common to these two amino acids. The molecular structure of $–NH_2$ and COOH moiety are not considerably affected by the composition of –R group since the bond length and bond angles remains roughly constant whatever the amino acids studied. Only –N-CHR-C-group is readily affected by the nature of –R. The presence of the hydroxy group in the side chain of threonine leads a level of complexity not found in simple amino acids. Tyrosine is one of the two aromatic amino acids its side chain consists of a $–CH_2$ group bonded in Para position on a phenol ring. The theoretical calculations have revealed that the most stable form of tyrosine is one of the conformer in which the phenyl ring pointed towards the amino group on the other hand the less stable conformer has phenyl ring pointed towards the carbonyl group. Calculated geometrical parameter are compared with experimental values and with the results reported by earlier workers in Table 2. In Table 2 the geometric parameters calculated for tyrosine in gas phase are compared with calculated gas phase values. Total electronic energies of hydroxy amino acids (threonine and tyrosine) using RHF and B3LYP methods by employing 6-311++G(d,p) and aug-cc-pVDZ basis sets with and without zero point energy corrections (ZPC) are listed in Table 3.

Table 3: Total electronic energies (a.u.) of threonine and tyrosine using RHF and B3LYP methods by employing 6-311++G(d,p) and Aug-cc-Pvdz basis sets without and with zero point energy corrections respectively.

Methods/Basis Set		threonine		tyrosine	
		Without ZPC	With ZPC	Without ZPC	With ZPC
RHF	6-311++G(d,p)	-435.897448	-435.745532	-626.445840	-626.238621
	Aug-cc-pVDZ	-435.838784	-435.687258	-626.368249	-626.161247
B3LYP	6-311++G(d,p)	-438.425267	-438.284285	-630.207668	-630.014870
	Aug-cc-pVDZ	-438.350257	-438.211864	-630.111793	-629.919145

The total electronic energy of threonine with ZPC and without ZPC at DFT/B3LYP level of theory with 6-311++G(d,p) and aug-cc-pVDZ basis set are found to be minimum than the corresponding total electronic energy with ZPC at the RHF level of theory with corresponding basis sets. Further, if we go from lower basis set i.e 6-311++G(d,p) to higher basis set i.e aug-cc-pVDZ yields a decrease in the total electronic energy. From Table 3 it is clear that tyrosine molecule follows similar trend like threonine molecule.

3.2 Dipole moments, Polarizabilities and Rotational constants

The calculated dipole moments, polarizabilities and rotational constants of hydroxy amino acids (threonine and tyrosine) at the RHF and B3LYP levels of theory with 6-311++G(d,p) and Aug-cc-pVDZ basis sets in the gas phase are listed in Tables 4 and 5 respectively.

Table 4: Dipole moment (Debye) and Polarizabilities of threonine and tyrosine using RHF and B3LYP methods by employing 6-311++G(d,p) and Aug-cc-pVDZ basis sets respectively.

Molecules	Dip./Pol.	RHF		B3LYP	
		6-311++G(d,p)	Aug-cc-pVDZ	6-311++G(d,p)	Aug-cc-pVDZ
Threonine	μ	3.0626	2.9576	3.0190	2.9138
	α_{xx}	66.348	70.361	77.282	81.368
	α_{xy}	1.019	0.538	0.855	0.367
	α_{yy}	60.826	63.770	69.905	72.896
	α_{xz}	3.354	3.090	3.542	3.370
	α_{yz}	2.738	2.416	2.420	2.150
	α_{zz}	54.314	57.251	61.074	64.213
Tyrosine	μ	0.3878	0.2693	0.5796	0.3960
	α_{xx}	145.631	152.802	169.309	176.584
	α_{xy}	0.212	0.971	1.587	2.569
	α_{yy}	114.238	118.874	125.986	130.551
	α_{xz}	-8.083	7.923	-9.719	9.436
	α_{yz}	4.701	-4.697	4.014	-4.529
	α_{zz}	77.972	82.937	82.948	88.254

Table 5: Rotational constants of threonine and tyrosine using RHF and B3LYP methods by employing 6-311++G(d,p) and Aug-cc-pVDZ basis sets respectively.

Molecules	Rotational constants	RHF		B3LYP	
		6-311++G(d,p)	Aug-cc-pVDZ	6-311++G(d,p)	Aug-cc-pVDZ
Threonine	A	3.20027	3.19212	3.12809	3.12083
	B	1.51074	1.50992	1.47628	1.47373
	C	1.31433	1.30953	1.29498	1.29169
Tyrosine	A	2.32658	2.32260	2.27362	2.27376
	B	0.34455	0.34323	0.34190	0.34036
	C	0.31435	0.31386	0.31098	0.31089

Since there has been no experimental attempt to determine microwave spectra of gaseous threonine and tyrosine, the theoretically predicted rotational constants may be useful in searching gas phase conformers of threonine and tyrosine with microwave spectroscopy. Similarly, theoretically predicted dipole moments may assists experimentalist on the permanent dipole measurement. At both level of the theories RHF and B3LYP when we go from lower basis set to higher basis set, the dipole moments slight decreases in all the cases. This decrease is more at the B3LYP procedure which includes electron correlation than the RHF method. The polarizability tensor components also show a change in going from 6-311++G(d,p) basis set to Aug-cc-pVDZ basis set at both level of theories. The xx, xy, yy and zz components of tyrosine increases while xz and yz component decreases at both level of theory by changing the basis set from lower level to higher level. In case of threonine xx, yy and zz component increases while xy, xz and yz component decreases. But they do not follow any regular pattern. The change in polarizability tensors xx, yy and zz are more pronounced in all cases considered here at both level of theory.

3.3 Vibrational characteristics

Vibrational frequencies of hydroxy amino acids (threonine and tyrosine) obtained through the IR spectrum are analyzed with the help of calculated frequencies using ab initio and DFT calculations. The Infrared spectrum has been taken between 400-4000 cm^{-1} and is shown in Fig. 3 for tyrosine.

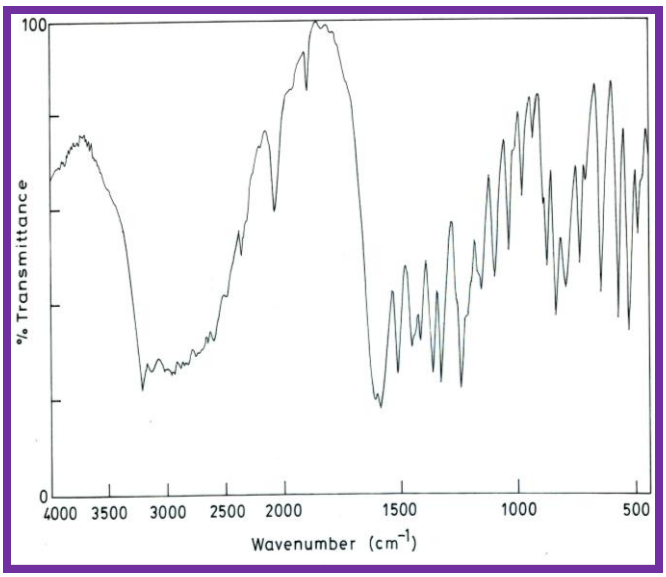

Fig 3: IR spectrum of tyrosine molecule.

There are seventeen (17) and twenty four (24) atoms in the gaseous threonine and tyrosine molecule respectively

and these two molecules having 45 and 66 vibrational modes respectively. But, the vibrational modes below 400 cm^{-1} are not listed in the tables for the sake of simplicity. The vibrational mode greater than 400 cm^{-1} i.e thirty seven (37) vibrational modes for threonine and fifty five (55) vibrational modes for tyrosine are listed in the corresponding tables. Tables 6 and 7 shows the observed and theoretically calculated vibrational frequencies at the RHF and DFT level of theory by employing 6-311++G(d,p) and aug-cc-pVDZ basis sets for the hydroxy amino acids threonine and tyrosine respectively. The vibrational assignments for corresponding vibrational frequencies are also included in these tables (Tables 6 and 7).

Table 6: Calculated and experimental fundamental frequencies (cm^{-1}) of threonine.

S. No.	Calculated frequencies				Expt.[a] (IR)	Ref.[b]	Ref.[c]	Assignments
	RHF		B3LYP					
	6-311++G (d,p)	Aug-cc-pVDZ	6-311++G(d,p)	Aug-cc-pVDZ				
1	485 (3)	482 (2)	450 (4)	448 (4)	447	-	-	Φ
2	511 (69)	504 (98)	475 (28)	473 (29)	491	-	-	Φ
3	540 (97)	531 (61)	545 (22)	539 (32)	-	-	-	Tw of OH
4	583 (109)	579 (88)	574 (164)	558 (129)	560	-	-	Φ
5	678 (68)	672 (65)	630 (58)	625 (53)	-	-	-	Φ
6	705 (31)	700 (29)	659 (34)	654 (32)	-	-	-	Φ
7	794 (46)	792 (42)	728 (28)	725 (27)	747	-	-	Φ
8	909 (94)	908 (75)	830 (76)	828 (61)	-	-	-	WNH_2
9	946 (11)	946 (12)	869 (24)	870 (25)	871	-	-	δNH_2
10	981 (80)	982 (75)	892 (77)	890 (65)	-	-	-	Φ
11	1022 (1)	1020 (1)	937 (1)	937 (1)	932	-	-	Φ
12	1110 (22)	1105 (20)	1026 (25)	1020 (21)	-	-	-	δCH_3
13	1184 (26)	1186 (42)	1076 (53)	1079 (55)	1040	-	1071	Tw CC
14	1192 (59)	1187 (43)	1104 (27)	1098 (23)	1093	-	-	Tw CC
15	1265 (131)	1262 (120)	1135 (154)	1136 (133)	-	-	-	υ CC
16	1284 (120)	1280 (102)	1162 (113)	1160 (110)	1185	-	1165	Tw CH
17	1337 (122)	1332 (134)	1221 (49)	1215 (59)	-	-	-	$Tw\ NH_2$
18	1379 (17)	1378 (17)	1257 (21)	1251(18)	1246	-	-	Φ
19	1423 (11)	1419 (5)	1301 (3)	1293 (1)	-	-	1253	Tw CN
20	1468 (24)	1461 (16)	1336 (24)	1328 (19)	1318	-	-	δ CH
21	1495 (13)	1486 (10)	1369 (11)	1358 (10)	1347	-	1354	δ CH
22	1531 (9)	1519 (10)	1401 (5)	1385 (8)	1383	-	-	$W\ CH_3$
23	1558 (56)	1546 (60)	1415 (23)	1410 (25)	-	-	-	Φ + δ CC
24	1567 (43)	1562 (41)	1430 (55)	1416 (59)	1418	-	1402	δ CH
25	1607 (8)	1590 (10)	1489 (6)	1466 (6)	1457	-	-	δCH_3
26	1620 (2)	1601 (2)	1501 (5)	1476 (5)	-	-	1478	δCH_3
27	1785 (73)	1767 (69)	1641 (77)	1623 (69)	1626	-	1656	$W\ NH_2$
28	1994 (429)	1979 (396)	1807 (304)	1798 (282)	1651	-	1804	υ C=O of COOH
29	3152 (19)	3154 (22)	2999 (20)	3004 (20)	2873	-	-	υ_s C-H
30	3163 (32)	3167 (30)	3022 (23)	3028 (23)	2978	2974	-	υ_s C-H of CH_3
31	3221 (23)	3232 (20)	3078 (2)	3090 (1)	2998	2912	2840	υ_s C-H
32	3240 (25)	3250 (26)	3094 (35)	3106 (36)	-	2989	2862	υ_s C-H_2
33	3258 (46)	3266 (49)	3109 (23)	3121 (23)	-	-	2914	υ_{as} C-H_2
34	3760 (15)	3754 (13)	3516(19)	3503 (17)	3026	3353	3397	υ_s N-H_2
35	3845 (26)	3843 (24)	3611 (22)	3602 (21)	3169	3505	3469	υ_{as} N-H_2
36	4117 (148)	4112 (138)	3712 (61)	3699 (55)	-	3600	3758	υ O16-H17
37	4123 (68)	4117 (62)	3762 (84)	3745 (79)	-	3686	3705	υ O6-H7 of COOH

[a] Ref.[12], [b] Ref.[10], [c] Ref.[11]

Vibrational spectra recorded in a nujoll mull. Numbers shown in the parenthesis correspond to the IR intensities. υ=Stretching, υ_{as}=Asymmetric stretching, υ_s=Stretching, δ=bending, δ_s= Symmetric bending, δ_{as}= Asymmetric bending, W = wagging, Sc= scissoring, T_w = twisting T=torsion, Φ=deformation in molecule

Table 7: Calculated and experimental fundamental frequencies (cm^{-1}) of tyrosine.

S. No.	Calculated frequencies				Exptl.[a] (IR)		Ref.[a]	Assignments
	RHF		B3LYP		In nujoll mull (liquid)	KBr (Solid)		
	6-311++G (d,p)	Aug-cc-pVDZ	6-311++G (d,p)	Aug-cc-pVDZ				
1	424 (9)	421 (9)	389 (9)	387 (9)	-	-	416	δ C-C
2	457 (5)	456 (7)	420 (2)	421 (2)	-	-	448	δ C-C
3	462 (8)	461 (4)	428 (10)	426 (8)	450	-	454	δ C-C
4	522 (17)	520 (18)	486 (14)	484 (16)	-	-	524	δ C-C
5	545 (11)	542 (11)	500 (10)	500 (10)	500	491	571	δ C-C
6	599 (9)	598 (10)	550 (18)	550 (18)	550	528	600	δ C18-C20
7	623 (160)	623 (143)	597 (125)	600 (111)	600	575	680	δ C-C-N
8	701 (.6)	699 (0.7)	653 (6)	648 (7)	-	-	712	δ C-C ring
9	717 (15)	712 (14)	657 (3)	652 (1)	-	-	755	δ C-C ring
10	767 (12)	766 (10)	709 (12)	710 (11)	680	648	761	δ O22-H24
11	806 (4)	806 (4)	738 (5)	745 (4)	750	712	809	υ C-COOH
12	851 (36)	851 (32)	787 (35)	787 (31)	780	739	833	δ N-H
13	907 (18)	903 (20)	812 (16)	812 (8)	800	798	851	δ N-H
14	909 (17)	907 (12)	834 (54)	834 (57)	850	-	873	δ C-H ring
15	927 (75)	924 (67)	843 (43)	844 (35)	860	840	883	δ C-H ring
16	942 (84)	943 (70)	863 (74)	864 (64)	877	877	898	δ C-H ring
17	955 (12)	952 (15)	879 (1)	878 (0)	896	-	920	δ C9-C5
18	1023 (38)	1022 (35)	923 (20)	927 (21)	-	-	1015	δ C-H ring
19	1061 (2)	1054 (4)	952 (10)	954 (8)	939	940	1035	δ C-H ring
20	1081 (38)	1078 (7)	967 (1)	968 (1)	-	-	1041	υ C3-C5
21	1087 (3)	1081 (39)	989 (15)	987 (15)	983	984	1078	δ C-H
22	1103 (2)	1100 (2)	1030 (1)	1023 (1)	1041	1041	1120	δ C-C
23	1171 (29)	1176 (22)	1114 (27)	1110 (6)	1099	1100	1181	υ C3-N1
24	1199 (20)	1196 (18)	1122 (152)	1123 (163)	-	-	1206	δ OH-CH
25	1229 (51)	1229 (49)	1146 (123)	1144 (52)	1111	-	1220	δ C-C ring
26	1264 (122)	1262 (127)	1159 (31)	1152 (83)	1155	1157	1260	δ O20-H21
27	1283 (60)	1276 (51)	1188 (157)	1186 (99)	-	-	1301	δ C-H ring
28	1291 (138)	1286 (132)	1197 (8)	1189 (59)	1174	-	1321	υ C-C
29	1312 (2)	1312 (2)	1227 (1)	1225 (2)	1215	-	1326	υ C-C
30	1351 (80)	1353 (72)	1263 (9)	1253 (17)	1244	1244	1338	δ O-H of COOH
31	1382 (86)	1376 (80)	1276 (116)	1273 (105)	-	-	1374	υ C18-O20
32	1385 (68)	1379 (69)	1297 (2)	1291 (2)	-	-	1367	δ C-H
33	1424 (11)	1418 (11)	1332 (30)	1330 (22)	-	-	1431	υ C5-O22
34	1460 (49)	1456 (34)	1349 (14)	1343 (11)	-	-	1481	δ C3-H4
35	1472 (19)	1464 (20)	1359 (8)	1348 (14)	1331	1330	1490	δ C-H
36	1506 (15)	1497 (17)	1361 (17)	1369 (17)	1363	1364	1511	δ C3-H
37	1556 (19)	1546 (22)	1420 (5)	1406 (8)	-	-	1526	δ CH_2
38	1583 (22)	1580 (24)	1466 (19)	1462 (14)	1415	1416	1579	υ C-C
39	1614 (5)	1595 (5)	1488 (8)	1469 (12)	1452	1453	1655	δ CH_2
40	1676 (138)	1672 (135)	1543 (112)	1538 (111)	1512	1514	1681	υ C-C ring
41	1775 (20)	1780 (22)	1631 (15)	1636 (17)	-	-	1734	υ C-C ring
42	1804 (61)	1794 (26)	1653 (50)	1652 (27)	1587	1589	1774	υ C-C ring
43	1809 (35)	1806 (58)	1670 (32)	1657 (44)	1612	-	1852	υ C-C ring + δ NH_2
44	1990 (460)	1976 (423)	1803 (342)	1794 (315)	1701	1902	1960	υ C=O
45	3180 (24)	3186 (24)	3031 (19)	3040 (19)	-	-	3191	υ CH_2 +CH ring
46	3223 (8)	3230 (8)	3069 (7)	3079 (7)	-	-	3229	υ CH_2 +CH ring
47	3235 (23)	3243 (24)	3084 (15)	3096 (15)	-	2958	3248	υ CH_2
48	3306 (25)	3317 (24)	3148 (22)	3159 (22)	-	-	3344	$υ_{as}$ C-H ring
49	3313 (10)	3322 (10)	3157 (9)	3165 (8)	-	-	3346	$υ_{as}$ C-H ring
50	3350 (16)	3357 (15)	3191 (9)	3198 (12)	-	-	3368	$υ_s$ C-H ring
51	3352 (1)	3360 (2)	3195 (2)	3200 (1)	3206	3208	3399	$υ_s$ C-H ring
52	3735 (7)	3727 (6)	3495 (4)	3481 (3)	-	-	3775	$υ_s$ NH_2

53	3812 (10)	3807 (9)	3573 (8)	3564 (7)	-	-	3887	υ_{as} NH_2
54	4112 (141)	4106 (132)	3748 (67)	3736 (63)	-	-	3930	υ_s O-H of COOH
55	4189 (107)	4181 (103)	3836 (68)	3816 (66)	-	-	4048	υ_s O-H

[a] Ref.[22]

Vibrational spectra recorded in a nujoll mull. Numbers shown in the parenthesis correspond to the IR intensities. υ=Stretching, υ_{as}=Asymmetric stretching, υ_s=Stretching, δ=bending, δ_s= Symmetric bending, δ_{as}= Asymmetric bending, W = wagging, Sc= scissoring, T_w = twisting T=torsion, Φ = deformation in molecule

As we know that scaling varies with method and basis sets, and different scaling factors 0.8929 in RHF[37] and 1.0167 in DFT[38] are recommended in the literature. Even with these scaling, the calculated frequencies do not match well with the observed ones for two reasons. One reason is of course that the calculations refer to gas phase where hydroxy amino acids are neutral while the observation refers to zwitterionic form of amino acids. Another reason may be the small differences between the calculated and the observed geometrical parameters. Therefore scaling has been not carried out, although there is a good agreement (within 100 cm^{-1}) between observed and calculated frequencies. Also, the agreement is very well with other earlier reported vibrational frequencies for threonine and tyrosine. The three different spectral regions of the infrared spectrum of these two hydroxy amino acids have been discussed below.

4000-2800 cm^{-1} region

In case of threonine the O-H stretching of carboxylic acid group and O-H stretching of alcohol group have been found at the 3745, 3699 and 1798 cm^{-1} at the DFT/B3LYP/aug-cc-pVDZ level of theory (See Table 6). Our calculated stretching vibrational modes for O-H (carboxylic) and O-H (alcohol) are well agreed with earlier reported corresponding stretching vibrational modes by B. Lakard.[10] The stretching vibrational modes for NH_2, CH_3, CH, and CN are also in a good agreement with earlier reported corresponding stretching vibrational modes.[10, 12] The NH_2 stretching vibrations present in all amino acids. The asymmetric stretching of NH_2 was observed at 3026 and 3169 cm^{-1} by Silva et al[12], our calculated stretching modes of NH_2 has been found at 3602 and 3503 cm^{-1} are higher than the observed ones by Silva et al but well agreed with theoretically calculated by B. Lakard.[10] The CH vibration modes has been found from 3121 cm^{-1} to 3004 cm^{-1} at DFT/B3LYP/aug-cc-pVDZ level of theory and in good agreement with observed ones 2882 cm^{-1} and 2932 cm^{-1} by Silva et al as C-H stretching modes. The stretching vibrational modes have been found at the 3736, 3816 and 1794 cm^{-1} for the corresponding O-H stretching of carboxylic acid group and O-H stretching of alcohol group at the DFT/B3LYP/aug-cc-pVDZ level of theory and well agreed with earlier reported theoretical calculation (See Table 7) at the CASSCF level of theory by Samuels et al.[22] of tyrosine molecule. In the observed IR spectrum in liquid and solid phase the stretching vibrational modes have been predicted at the 3930 cm^{-1} for O-H of COOH and 4048 cm^{-1} O-H for alcohol. The theoretically calculated stretching modes for both O-H groups agree well with observed ones. The asymmetrical NH_3 stretching vibrational mode has been found at the 3564 and 3573 cm^{-1} at the B3LYP/aug-cc-pvdz and B3LYP/6-311++G(d,p) basis set. The Ramaekers et al[17] reported this asymmetrical NH_3 stretching vibrational mode at 3408 cm^{-1}. The symmetrical NH_3 stretching vibrational mode is almost 100 cm^{-1} lower than the asymmetrical stretching vibrational modes. The CH stretching vibrational modes may be divided into two groups, first is the aromatic C-H and second is aliphatic C-H stretching vibrational modes. The former C-H stretching vibrational modes found above the 3000 cm^{-1} and latter being below the 3000 cm^{-1}. The theoretically calculated values are in good agreement with observed ones.

2000-1000 cm^{-1} region

In threonine, the theoretically calculated C=O stretching vibrational mode at the DFT/B3LYP level of theory with 6-311++G(d,p) and aug-cc-pVDZ basis sets is found at the 1798 and 1807 cm^{-1} respectively and well agreed with C=O stretching mode reported by B. Lakard [10] (See Table 6). The bending mode of the CH_3 has been found at the 1466 and 1476 cm^{-1} at the DFT/B3LYP level of theory with aug-cc-pVDZ basis set. Bending vibrational mode of CH group has been found in between 1385 cm^{-1} to 1215 cm^{-1}. The most prominent band observed in the 2000-1000 cm^{-1} region is C=O stretching vibrational mode in tyrosine. In liquid phase this bands originate at 1701 cm^{-1} while in solid phase at 1902 cm^{-1}. The theoretically calculated C=O stretching vibrational mode is found at the 1794 and 1803 cm^{-1} with 6-311++G(d,p) and aug-cc-pVDZ respectively at the DFT/B3LYP level of theory. The calculated and observed C=O stretching vibrational mode is well agreed with earlier reported results.[17, 22] The C-C stretching vibrational mode originates at around 1500 cm^{-1}, while C-H stretching mode originates in between 1500-1000 cm^{-1}. The bending vibrational mode of O-H group of COOH has been found at 1253 cm^{-1} at DFT/B3LYP level of theory, whereas the vibrational mode of O-H group of alcohol has been found at 1155 cm^{-1}.

1000-400 cm^{-1} region

The lower region of the IR spectrum of threonine and tyrosine molecules is complex in nature. The vibrational bands originates in this region are basically due to deformation in the skeleton of the molecule, deformation in the different functional groups, torsions in the different groups and mixing of different type of motions in the molecule.

4 CONCLUSION

The molecular structures of threonine and tyrosine molecules in its neutral form have been optimized using ab initio quantum mechanical calculations and DFT level of theory. We have determined the molecular structures, Dipole moments, Polarizabilities, Rotational constants and Vibrational frequencies of these selected two hydroxy amino acids. It is clear that the geometrical parameters obtained for these two amino acids are basis sets dependent. The addition of polarization function leads to longer bond length and shorter bond angles. The changes in different stretching vibrational modes of O-H groups have been analyzed from threonine to tyrosine.

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