UV-VIS, NMR AND FT-IR SPECTRA OF TAUTOMERS OF VITAMIN C. EXPERIMENTAL AND DFT CALCULATIONS

HOSSEIN A. DABBAGH, FATEMEH. AZAMI, HOSSEIN FARROKHPOUR,
ALIREZA NAJAFI CHERMAHINI*

Department of Chemistry, Isfahan University of Technology, 8415483111 Isfahan, Iran

ABSTRACT

Experimental and computational analysis of spectroscopic parameters of L-ascorbic acid (vitamin C) and its tautomers was investigated by Density Functional Theory (DFT) using B3LYP method. The optimized geometries and calculated vibrational frequencies were evaluated. The latter was compare with those of experimental values. The $\lambda_{\max}$ values of L-ascorbic acid were found 259 (at pH = 3) experimentally, 238 and 247 nm calculated in the gas and water phase at TD-DFT methods, respectively. The experimental order of $\nu(\text{O-H})$ modes was obtained at $\nu(\text{O}_3\text{-H}_3) > (\text{O}_6\text{-Ho}_6) > (\text{O}_2\text{-H}_2) > (\text{O}_5\text{-Ho}_5)$. The theoretical orders were calculated at $\nu(\text{O}_6\text{-Ho}_6) > (\text{O}_2\text{-H}_2) > (\text{O}_5\text{-Ho}_5) > (\text{O}_3\text{-H}_3)$ (and $\nu(\text{O}_3\text{-H}_3) > (\text{O}_5\text{-Ho}_5) > (\text{O}_6\text{-Ho}_6) > (\text{O}_2\text{-H}_2)$), respectively, in the gas phase and aqueous phase. The highest wave number of tautomers (*S,Z*)-3,5,6-trihydroxy-2-oxohex-3-enoic acid (**2**), (*2Z,4E*)-2,3,5,6-tetrahydroxyhexa-2,4-dienoic acid (**3**), (*R*)-2-((*S*)-1,2-dihydroxyethyl)-4,5-dihydroxyfuran-3(2H)-one (**4**) was calculated for the stretching modes $\text{O}_6\text{-Ho}_6$, $\text{O}_3\text{-H}_3$, $\text{O}_6\text{-Ho}_6$ bonds, respectively, in the gas phase. The experimental and theoretical calculations of proton NMR predicted H_3 as the most deshielded proton.

Keywords: Vitamin C, tautomers, UV-VIS, NMR, FT-IR

INTRODUCTION

Ascorbic acid (AA) is one of the interesting and well known acids in nature which is synthesized from D-glucose or D-galactose [1]. Generally, AA is used in pharmaceutical, chemical, cosmetic and food industry due to its bioactivity and as antioxidant [2]. The lack of ascorbic acid leads to scurvy [3]. Ascorbic acid is known to increase the accessibility and absorption of iron from non-heme iron sources [4]. Vitamin C has been demonstrated to kill HIV-positive cells and help HIV-positive individuals by increasing the immune system [5]. Vitamin C significantly decreases the adverse effect of reactive species such as reactive oxygen and nitrogen that can cause oxidative damage to macromolecules such as lipids, DNA and proteins[6]. Ascorbic acid radical produced by the anodic oxidation of ascorbic acid was detected using UV spectroscopy [7]. Y. Dimitrova, studied the hydrogen-bonded system formed between L-ascorbic acid and five water molecules [8]. R. A. Yadav et al. studied the neutral L-ascorbic acid molecule and its cation and anion [9]. J.N. Patricia observed benzene formation in certain beverages containing benzoic and ascorbic acids under heat and UV exposure [10]. D. Giuntini et al. investigated ascorbic acid content in tomato fruits under UV-B radiation [11]. A method for the simultaneous measurement of ascorbic acid and uric acid in human seminal plasma has been described using HPLC with UV detector [12]. A.R. Wedina et al. applied solid-phase UV spectrophotometer technique to measure ascorbic acid[13]. M. Surmeian described new method for resolving three-component drug mixture[14]. H.F. Ji and E. L. Shen studied quenching mechanisms of triplet state riboflavin by vitamin C [15-17]. J.R. Juhasz et al. studied the effects of conformation on the acidity of L-ascorbic acid [18]. The role of the ascorbic acid concentration in the formation of gold nanorods was studied by UV-vis absorption and transmission electron microscopy[19]. I. Georgieva et al. reported DFT study of four low-energy neutral, four anion and two dianion conformers of AA [20]. Natural abundance ^{17}O -NMR spectra of L-ascorbic acid were recorded in aqueous solution as a function of pH by A. Ruchmann et al. [21]. M. B. Damian et al. studied two stable conformers of L-ascorbic acid and its fully oxidized form [22]. Ab initio conformational studies were performed on all local and global minima of PES at the MP2/6-311G (d,p) level of theory [23]. Gino A. and co-workers reported the correlation between ascorbate anion (AH2) and its oxidation products [24].

The stability of Vitamin C is under scrutiny. It may be decompose at room temperature when is expose to air or during cooking process. It is very sensitive to heat, alkali, oxygen, light and even moisture [1]. Traces of copper (II) and iron (III) can deeply accelerate the oxidation in aqueous solution [2]. The degradation of vitamin C may coincide with isomerization, tautomerism or racemization.

In this report, experimental and computational analysis of spectroscopic parameters (FT-IR, UV, ^1H -NMR and ^{13}C -NMR) of L-ascorbic acid and three

(potential side products) of its tautomers [(*S,Z*)-3,5,6-trihydroxy-2-oxohex-3-enoic acid (**2**), (*2Z,4E*)-2,3,5,6-tetrahydroxyhexa-2,4-dienoic acid (**3**), (*R*)-2-((*S*)-1,2-dihydroxyethyl)-4,5-dihydroxyfuran-3(2H)-one (**4**)] (Fig. 1) was investigated by Density Functional Theory (DFT) using B3LYP method. The tautomerization of vitamin C to symmetrical tautomers may result in VC racemization during storage or cooking. The UV, NMR and IR of the mixture may help measure the tautomers contents. The optimized geometries and calculated vibrational frequencies are evaluated and compared with those of experimental values.

COMPUTATIONAL METHOD

L-Ascorbic acid and its tautomers were fully optimized at the B3LYP/6-31++G (d,p) level of theory. The FT-IR and UV-Vis spectra were computed at the same level of theory using the GAUSSIAN 03 program [25]. The theoretical electronic absorption spectra were calculated using TD-DFT methods. Calculations of ^1H -NMR and ^{13}C -NMR were performed at the GIAO/B3LYP/6-31+G(2d, p) levels of theory (at TMS/GIAO/B3LYP/6-311+G(2d,p)). The calculations were performed for two solvent (DMSO and water using the polarizable continuum model (PCM) [26-29].

Fig. 1. L-ascorbic acid (**1**) and its proton tautomers H_2 (**2**), H_5 (**3**) and H_3 (**4**).

EXPERIMENTS

L-Ascorbic acid [(4R-5S)-AA] compound (**1**) was purchased from Acros Organics Chemical Co. It was used as such without further purification. The IR spectra were recorded in KBr pellets using a JASCO FT-IR-680 Plus spectrometer in the spectral range 400–4000 cm^{-1} . ^1H -NMR and ^{13}C -NMR spectra in D_2O and DMSO were recorded by a Bruker Avance 400 spectrometer. UV-Vis spectra in deionized water were recorded by a UVD-2950 LABOMED, INC spectrophotometer.

RESULTS AND DISCUSSION

Ascorbic acid (**1**) is known to be unstable molecule which easily undergoes isomerization, decomposition and/or racemization. In this report, spectroscopic analysis (UV-Vis, NMR and FT-IR) of vitamin C tautomers **2-4** was investigated. The structure, numbering and proton tautomers H_2 (**2**), H_5 (**3**) and H_3 (**4**) of L-ascorbic acid are presented in Figure 1. The relative energy of **1**, **2**, **3** and **4** was calculated 0, 3.8, 5.5 and 5.6 kcal/mol at B3LYP/6-31++G(d,p) level of theory, respectively. The tautomerization of **4** produces very unstable isomer (dihydroxyfuran-3-one derivative) with relative energy of 24.1 kcal/mol was excluded from this study.

4. 1 The UV-visible spectrum

The observed and calculated visible absorption maxima of the compounds **1-4** are given in Table 1. The single-excitation configuration interaction time-dependent density functional theory (TD-DFT) calculations of molecular orbital geometry in the gas and water phase show that visible absorption maxima of L-ascorbic acid correspond to electron transition from HOMO to LUMO. The maximum amount of f values with highest % was obtained for these electronic transitions at λ_{max} . The λ_{max} value for L-ascorbic acid was found at 238 nm in the gas phase. This value was increased to 248 nm in the water (solvent effects). The calculated λ_{max} values in the gas phase for the compounds **2**, **3**, **4** were found at 342, 309 and 273 nm, respectively. The greater λ_{max} value for **2** is attributed to extra conjugation. Based on the f values collected in Table 1, it is evident that absorption maxima of **2**, **3** and **4** correspond to the electron transition from HOMO to LUMO.

Table 1. The energy and molar absorption coefficients of experimental absorption bands and the electronic transitions calculated with TD-DFT method for L-ascorbic acid and its tautomers.

Compound (1) in gas phase			
The most important orbital excitations	(nm) λ	E (eV) ^a	f^b
H→L ^c (27%) ^d	247.4	5.012	0.0181
H→L+1 (+45%)	247.4	5.012	0.0181
H→L+2 (25%)	247.4	5.012	0.0181
H-1 →L (18%) [*]	238.02	5.2089	0.1075
H-1 →L+1	238.02	5.2089	0.1075
H→L (+41%) [*]	238.02	5.2089	0.1075
H→L+1	238.02	5.2089	0.1075
H→L+2	238.02	5.2089	0.1075
H-1 →L (+65%) [*]	234.08	5.2967	0.0267
H-1 →L+1	234.08	5.2967	0.0267
H→L	234.08	5.2967	0.0267
H→L+2	234.08	5.2967	0.0267
Compound (2) in gas phase			
The most important orbital excitations	(nm) λ	E (eV)	f
H-1→L (92%) [*]	399.67	3.1023	0.0001
H →L (80%) [*]	342.06	3.6246	0.1667
H-5 →L (70%) [*]	248.57	4.9879	0.0007
H-3 →L	248.57	4.9879	0.0007
H-2→L	248.57	4.9879	0.0007

H-1→L+2	248.57	4.9879	0.0007
Compound (3) in gas phase			
The most important orbital excitations	(nm) λ	E (eV)	f
H→L (80%) [*]	309.53	4.0055	0.1379
H→L+1	309.53	4.0055	0.1379
H-2 →L	262.18	4.7290	0.0508
H-1 →L (22%) [*]	262.18	4.7290	0.0508
H→L+1(65%) [*]	262.18	4.7290	0.0508
H-2 →L (52%) [*]	257.65	4.8121	0.0125
H-2 →L+1	257.65	4.8121	0.0125
H-1→L	257.65	4.8121	0.0125
H→L+1	257.65	4.8121	0.0125
H→L+2	257.65	4.8121	0.0125
Compound (4) in gas phase			
The most important orbital excitations	(nm) λ	E (eV)	f
H-1→L (78%) [*]	275.11	4.5068	0.0108
H-1→L+1	275.11	4.5068	0.0108
H→L	275.11	4.5068	0.0108
H →L+1	275.11	4.5068	0.0108
H-1 →L	273.24	4.5375	0.0767
H→L (55%) [*]	273.24	4.5375	0.0767
H→L+1	273.24	4.5375	0.0767
H→L+2	273.24	4.5375	0.0767
H →L	261.18	4.7471	0.0539
H→L+1 (30%) [*]	261.18	4.7471	0.0539
H→L+2 (47%) [*]	261.18	4.7471	0.0539
Compound (1) in water phase			
The most important orbital excitations	(nm) λ	E (eV)	f
H→L (%) +80) [*]	247.71	5.0052	0.2578
H→L+1 (%) +84) [*]	234.47	5.2878	0.0066
H→L+2	234.47	5.2878	0.0066
H-3 →L	222.23	5.5791	0.0014
H-2 →L (%) +92) [*]	222.23	5.5791	0.0014
Experimental of Compound (1) in H_2O			
	(nm) λ	E (eV)	ϵ
	259	4.79	0.95416

^a $E = hc/\lambda$

^b $f =$ calculated intensity

^c H = HOMO, L=LUMO

^{*} S.I. Gorelsky, SWizard program, <http://www.sg-chem.net>

The energy (eV) and selected HOMO-LUMO contours of L-ascorbic acid (**1**) in water and in gas phase are depicted in Figures 2 and 3. The energy of HOMO and LUMO for **1** was calculated -6.53 and -1.18 eV in aqueous phase and -6.67 and -1.17 eV in gas phase, respectively. The energies of the HOMO decrease in the gas phase and for LUMO is nearly the same for both phases. The energy gap (ΔE) for **1** was calculated 5.35 and 5.50 eV in water and gas phase, respectively. Larger value of the energy gap implies high kinetic stability and low chemical reactivity. This is in good agreement with previous report that vitamin C is unstable in water [30]. The energy of HOMO and LUMO was calculated -7.11 and -3.11 eV for **2**, -6.46 and -1.95 eV for **3**, and -6.12 and

-1.26 eV for **4** in the gas phase, respectively. The energy gap (ΔE) for **2**, **3** and **4** was calculated 4.0, 4.51 and 4.86 eV in gas phase, respectively, which is smaller than that of vitamin C. The energy 4.79 eV obtained experimentally in H_2O complements the theoretical value (5.0 eV) calculated in water phase. The $\lambda_{max} = 259$ nm obtained experimentally in H_2O complements 248 nm value calculated in water phase.

The most important electronic transition (HOMO-LUMO) was calculated 41, 80, 80 and 55% at λ_{max} of 238.02, 342.06, 309.53 and 273.24 nm for **1**, **2**, **3** and **4**, respectively in the gas phase (Table 1). This value was calculated 80% at $\lambda_{max} = 247.71$ for **1**

in water phase (experimental $\lambda_{max} = 259$ nm). Positive values of the orbital contour are represented in brown (+0.02au) and negative values in green (-0.02) color.

Fig. 2. Energies (eV) and selected HOMO-LUMO contours of L-ascorbic acid (**1**) in water.

Fig. 3. Energies (eV) and selected HOMO-LUMO contours of L-ascorbic acid in gas phase.

4.2 Infrared analysis

The experimental and calculated infrared spectra of L-ascorbic acid in the gas and water are presented in Figure 4. The results of calculated vibrational modes and their experimental assignments are listed in Tables 2 and 3.

Fig. 4. Experimental (KBr, —) and calculated infrared spectra of L-ascorbic acid in the gas phase (---) and in water (---).

4.2.1 Tautomer 1 (experimental and computational)

O-H modes. L-Ascorbic acid (**1**) has four OH groups, two being attached to the ring and two on the side chain. The order of $\nu(O-H)$ modes experimentally are $\nu(O_3-H_3) > (O_6-H_{O6}) > (O_2-H_2) > (O_5-H_{O5})$ (in gas phase and $\nu(O_6-H_{O6}) > (O_5-H_{O5}) > (O_3-H_3) > (O_2-H_2)$ in water phase).

Experimentally, the O_6-H_{O6} stretching mode of the side chain of **1** was observed at 3410 cm^{-1} . The calculated values in the gas and water phase were found $3841, 3444\text{ cm}^{-1}$, respectively. The second stretching mode for the O_2-H_2 bond that attached to the ring were measured at $3316, 3812$, and 3375 cm^{-1} experimentally, calculated in the gas phase and in water phase, respectively. The third stretching mode O_5-H_{O5} in the side chain of **1** was observed experimentally at 3216 cm^{-1} . The calculated values in the gas and water were calculated at 3758 , and 3456 cm^{-1} , respectively. The fourth stretching mode for the O_3-H_3 bond (attached to the ring) was found at $3526, 3638$, and 3558 cm^{-1} experimentally, in the gas phase and water, respectively.

C=O mode. The stretching mode for the $C_1=O_1$ bond was found at $1753, 1862$, and 1789 cm^{-1} experimentally, calculated in the gas phase and in aqueous phase, respectively.

C=C mode. The stretching mode for the $C_2=C_3$ bond was found at $1672, 1771$, and 1705 cm^{-1} experimentally, calculated in gas phase and in water phase, respectively.

C-H modes. The calculated stretching mode for the C_5-H_5 in the side chain was found at 3003 and 2960 cm^{-1} in the gas phase and water phase, respectively. In addition the stretching mode for the C_4-H_4 bond in the ring was found at $2915, 3027$, and 2976 cm^{-1} experimentally, calculated in gas and water phase, respectively. The anti-symmetric stretching mode for the CH_2 group was found at $3030, 3110$, and 3076 cm^{-1} in KBr, calculated in the gas and water phase, respectively. The symmetric stretching mode for the CH_2 was found at $2744, 3056$ and 3016 cm^{-1} KBr, calculated in gas and water phase. The scissoring motion for the CH_2 group in the side chain was found at $1497, 1498$, and 1483 cm^{-1} experimentally, calculated in gas phase, and aqueous phase, respectively.

C-O modes. The stretching mode for the C_5-O_5 bond of the side chain was found at $1120, 1086$ and 1082 cm^{-1} experimentally, in gas and water phase, respectively. The stretching mode for the C_6-O_6 bond was found at $1076, 1044$, and 1060 cm^{-1} in KBr, calculated in gas and water phase. The stretching mode for the C_1-O_1 bond was found at 942 , and 1130 cm^{-1} , in gas and water phase, respectively. The stretching mode for the C_3-O_3 and C_2-O_2 groups of the ring was found at 1139 , and 1321 cm^{-1} in KBr, respectively.

C-C modes. The stretching mode for the C_1-C_2 was found 1126 , and 1418 cm^{-1} in the gas and water phase spectra, respectively. The stretching mode for the C_4-C_5 was found at 942 cm^{-1} in gas phase and the stretching mode for the C_5-C_6 was found at 869 cm^{-1} in KBr.

Table 2. Experimental (KBr) and calculated vibrational frequencies (cm⁻¹) of L-ascorbic acid (1) in the gas and water at the B3LYP/6-31++G(d,p) level of theory.

Exp.(ref. 9)	Gas	Water	Assignment	Exp.	Gas	Water	Assignment	Exp.	Gas	Water	Assignment ^a
3410	3841	3444	$\nu_{\text{O6-H O6}}$	1363	1386	1392	ω_{CH2} ω_{CSH5}	988	862	-	ρ_{CH2} δ_{SC5C6H6} $\nu_{\text{C5-O5}}$
								755			δ_{C4C5}
3316	3812	3375	$\nu_{\text{O2-H2}}$	1221	1374	1371	ρ_{CSH5} ρ_{C4H4}	681	780 714	715	α_{ring}
3216	3758	3456	$\nu_{\text{O5-H O5}}$		1357	-	δ_{SH6C6O6}	720	751	760	$\gamma_{\text{C1-O1}}$ γ_{C2O2}
3526	3638	3558	$\nu_{\text{O3-H3}}$		1343	1298	δ_{SC3O3H3}	628	681 493	691	$\nu_{\text{C1-C2}}$ δ_{SC1OrC4}
3030	3110	3076	$\nu_{\text{as CH2}}$	1274	1298	-	ρ_{C4H4} ω_{CSH5} δ_{SC2O2H2}				
2744	3056	3016	ν_{sCH2}	-	1251	-	ρ_{C4H4} ω_{CSH5} δ_{SC3O3H3}	565 592	643 470 621	626 464	ω_{C2C3} ω_{O3H3} ω_{C3C4}
2915	3027	2976	$\nu_{\text{C4-H4}}$	1139			$\nu_{\text{C3-O3}}$				
				1197			τ_{CH2}				
				1044 1025 820			ν_{ring}				
-	3003	2960	$\nu_{\text{C5-H5}}$	-	1185	3076 3016	$\delta_{\text{SC6O6H O6}}$		563 551	556.	ω_{C2O2} ω_{C3O3}
1753	1862	1789	$\nu_{\text{C1=O1}}$		1126	1418	$\nu_{\text{C1-C2}}$	447	528	262 253 211	$\tau_{\text{O5H O5}}$
1672	1771	1705	$\nu_{\text{C2=C3}}$	1120	1086 1055	1082	$\nu_{\text{C5-O5}}$ $\nu_{\text{C5-C6}}$	-	503	-	τ_{CH2}
				869			$\nu_{\text{C5-C6}}$				
1497	1498	1483.31	δ_{sCH2}	-	1064	1130	$\nu_{\text{C1-Or}}$ τ_{CH2}		334 364 356 313 297	351 128 56 -42	ω_{O2H2}
-	1446	1431	ω_{CSH5} $\omega_{\text{O5H O5}}$						266	163	$\omega_{\text{O6H O6}}$
1248	1432	1418	δ_{SC2O2H2} δ_{SC3O3H3}	1076	1044 1028	1060	$\nu_{\text{C6-O6}}$		202	75	ω_{CSO5}
1321			$\nu_{\text{C2-O2}}$	-	942		$\nu_{\text{C4-C5}}$				
1439	1396 1193	1298	δ_{SC3O3H3}						101	163	τ_{CH2} ω_{C6O6}
1388			$\delta_{\text{SC5O5H O5}}$								

^a ν =stretching, ω =wagging, out-of-plane ring bending, ρ = in plane ring, ν_{s} = symmetric stretching bending, δ =scissoring, angle bending, τ =twisting, ν_{as} =anti-symmetric stretching, α = in plane deformation, γ =out-of-plane deformation,

Table 3. Vibrational frequencies of compound 2, 3, 4 in the gas phase at B3LYP/6-311++G (d,p) level of theory.

Frequency			Assignment	Frequency			Assignment	Frequency			Assignment ^a
(2)	(3)	(4)		(2)	(3)	(4)		(2)	(3)	(4)	
3830	3764	3825	$\nu_{\text{O6-H O6}}$	1460			δ_{SH3O3C3} $\nu_{\text{O5-H3}}$			720	$\nu_{\text{C3-C4}}$
3760	3737	3787	$\nu_{\text{O5-H O5}}$	1390	1177		δ_{SC3C4H4}	838 280			$\nu_{\text{O5-H3}}$
	3777	3767	$\nu_{\text{O2-H2}}$		1264		δ_{SC3O3H3}	807			ω_{C1O1} ω_{C2C3}
3609	3831		$\nu_{\text{O3-H3}}$			1374	δ_{SC2O2H2}				
3583	3690	3790	$\nu_{\text{Or-H1}}$ $(\nu_{\text{O1-H1}})_{\text{s}}$		1125		$\nu_{\text{C1-O1}}$	340	763 46		ω_{C1O1} ω_{C2O2}

3212	3121	3075	ν_{C4-H4}	1388	1291 1328 1291	1119	$\delta_{SC10rH1}$ $(\delta_{SC101 H1})_5$	737	464	697 681 638	ρ_{C10r} ρ_{C202}			
				1384		1397	τ_{CH2} ω_{C5H5}							
				1367	1397	1436 1368 1356	$\omega_{CH2}, \omega_{C5H5},$ $\omega_{OSH O5}, \delta_{SC606H}$ O6							
3107	3118	3109	$\nu_{as CH2}$	1332			ω_{C5H5} ρ_{C4H4}	275 107 91 30	442 599 175 273 84 173	822 561	dc			
3041	3045	3019	$\nu_s CH2$	1282			$\delta_{SC505H O5}$ ω_{C5H5} $\rho_{OSH O5}$		558 773		$\delta_{SOrC101}$			
3041	3045	3019	$\nu_s CH2$	1244	1249		$\delta_{SC505H O5}$	700 253	706,333 309,237, 371,343		ω_{O3H3} ω_{C3C4}			
				1217	1182	$\delta_{SC202H2}$								
1827	1822		$\nu_{C1=O1}$			1263	$\delta_{SC5C4H4}$ ω_{C5H5} ρ_{C4H4}	658			ρ_{C3O3} ρ_{C4H4}			
1722			$\nu_{C2=O2}$	1188 1162	1187	1252 1382 1299	$\delta_{SC3C4H4}$ ω_{C4H4} ρ_{C101} ρ_{O1H1}	615		55 524	ω_{C3O3} ρ_{C4C5}			
		1700	$\nu_{C3=O}$			1053	$\delta_{SC3C4H4}$	383 424	486		ρ_{C6C5} ω_{OSH5}			
	1701		$\nu_{C4=C5 (S-Cis)}$			234	$\delta_{SC3C2O2}$							
1673			$\nu_{C3=C4}$	1036 1123	1016	1140 875	ν_{C5-O5}	340	486 522 495	148 94 73 60	dc ^b			
	1686	1463	$\nu_{C2=C3}$	1036	1016		ν_{C3-O3}		522 495	309 295	dc			
		1805	$\nu_{C1=C2}$	1010 1051	983 1046	1068 996	ν_{C6-O6}			382	ω_{C10r} ω_{O1H1}			
1491	1499	1495	δ_{SCH2}			927 1031	ν_{C4-Or} ρ_{C4C5}			353	ρ_{O1H1}			
1409	1424		$\delta_{SC5C4H4}$		854		ω_{C4H4} ω_{C5O5}			273 341 314	ω_{O1H1} ω_{C1C2} ω_{O2H2}			
	1408		$\delta_{SC202H2}$	877 859	668 872 854 130		dc			238 450	ω_{C4C5} $\omega_{OSH O5}$			
1409	1365		$\delta_{SO6C6H6(1)}^c$								291 296	495 486	511	$\omega_{O6H O6}$ τ_{CH2}
											972	784		ω_{C3C4} ω_{C1C2}

^a ν =stretching, ω =wagging, out-of-plane ring bending, ρ =in plane ring, ν_s =symmetric stretching bending, δ_s =scissoring, angle bending, τ =twisting, ν_{as} =anti-symmetric stretching, α =in plane deformation, γ =out-of-plane deformation.

^b dc=deformation of compound.

4. 2. 2 Tautomers of 2, 3, and 4 (gas phase)

The O_6-H_{O6} stretching mode **2**, **3** and **4** was calculated at 3830, 3764, and 3825 cm^{-1} , respectively. The second stretching mode for the O_5-H_{O5} bond was calculated at 3760, 3737, and 3787 cm^{-1} for tautomers **2**, **3** and **4**, respectively. The third stretching mode O_3-H_3 for **2** and **3** tautomers appeared at 3609 and 3831 cm^{-1} . The fourth stretching mode for the O_2-H_2 bond was calculated at 3777 and 3767 cm^{-1} for **3** and **4**, respectively. The fifth stretching mode for the O_r-H_{O1} bond calculated at 3583 and 3690 cm^{-1} **2** and **3** tautomers. The stretching

mode for the O_1-H_{O1} bond was calculated at 3790 cm^{-1} for **4**. There were two stretching modes for $O_5 \dots H_3$ hydrogen at 838 and 280 cm^{-1} for **3**.

C=O modes. The $\tilde{C}_1=O_1$ stretching mode of **2** and **3** were observed at 1827 and 1822 cm^{-1} , respectively. The second stretching mode for the $C_2=O_2$ bond appeared at 1722 cm^{-1} for isomer **2**. The third stretching mode of $C_3=O_3$ bond was calculated at 1700 cm^{-1} for **4**.

C=C modes. The $C_2=C_3$ stretching mode of **3** and **4** appeared at 1686 and 1463 cm^{-1} , respectively. The second stretching mode for the $C_1=C_2$ bond was calculated at 1805 cm^{-1} for isomer **4**. The third stretching mode for the $C_3=C_4$

bond was found at 1673 cm⁻¹ for tautomer **2**. The C₄=C₅ stretching mode for **3** was observed at 1701 cm⁻¹, respectively.

C-H modes. The stretching mode C₄-H₄ for tautomers **2**, **3** and **4** were calculated at 3212, 3121 and 3075 cm⁻¹. The stretching mode C₅-H₅ of **2** and **4** was obtained at 2953 and 2960 cm⁻¹, respectively. The anti-symmetric stretching modes of CH₂ were found at 3107, 3118 and 3109 cm⁻¹ for **2**, **3** and **4**. The symmetric stretching mode of CH₂ was found at 3041, 3045, and 3019 cm⁻¹. The scissoring mode for the CH₂ was found at 1491, 1499, and 1495 cm⁻¹ for **2**, **3** and **4** isomers, respectively.

C-O modes. The C₄-O₄ stretching modes for **2**, **3** and **4** were observed at 1051, 983 and 996 cm⁻¹, respectively. The C₃-O₃ stretching modes for **2** and **3** were observed at 1036 and 1016 cm⁻¹. The C₅-O₅ stretching mode of **2**, **3** and **4** tautomers were observed at 1036, 1016 and 1140 cm⁻¹, respectively. The C₁-O₁

stretching mode of **3** was observed at 1125 cm⁻¹. The C₄-O_r stretching mode of **4** was observed at 972 cm⁻¹.

C-C modes. The C₃-C₄ stretching mode of **4** was observed at 720 cm⁻¹.

4.3 NMR spectrum

4.3.1 NMR spectrum L-ascorbic acid

¹H-NMR: The experimental proton chemical shifts in D₂O were found at H₄ = 4.8, H₅ = 3.9, H_{6a} = 3.6, H_{6b} = 3.6 ppm (Table 4). The experimental value in the DMSO and theoretical calculations in both gas and water phase predict that the proton H₃ the most deshielded chemical shift which complements the finding of reference 32. That L-Ascorbic acid is a weak dibasic acid (pK_a=4.25 and pK_{a2}=11.79), the monoanion forms at pH 4–5 by deprotonation from O₃-H₃ bond. The experimental chemical shifts of H_{O6} and H_{O5} in DMSO appeared at 4.9 ppm.

Table 4. ¹H-NMR and ¹³C-NMR spectrums of L-ascorbic acid (**1**): Experimental and calculated values at DFT levels of theory using B3LYP/6-311+G(2d,p) basis set in the gas and water phase.

¹ H-NMR (TMS B3LYP/6-311+G(2d,p) GIAO) (1)										
Δδ	Cal. Water	Δδ	Cal. DMSO	Δδ	Cal. Gas ^c	Δδ	Exp. (D ₂ O)	Δδ ^b	Exp.(DMSO) ^a	Assignment
-1.5	1.8	-1.2	2.1	-3.3	0.0-		-	1.5	4.9	H _{O6}
0.5	3.8	0.9	4.2	-0.3	3.0		-	1.5	4.9	H _{O5}
4.1	7.4	4	7.3	3.3	6.6		-	7.6	11.0	H ₃
2.1	5.4	2.1	5.4	1.6	4.9	1.2	4.8	0.8	4.2	H ₄
1.4	4.7	1.3	4.6	1	4.3	0.3	3.9	0.3	3.7	H ₅
0.7	4.0	0.6	3.9	0.7	4.0	0	3.6	0.1	3.5	H ₆
0	3.3	0	3.3	0	3.3	0	3.6	0	3.4	H ₆
1.6	4.9	2.4	5.7	-0.1	3.2		-	5	8.4	H ₂
¹³ C-NMR [TMS B3LYP/6-311+G(2d,p) GIAO] (1)										
Δδ		Cal. Water		Δδ	Cal. gas		Δδ		Exp.(DMSO)	Assignment
110.4		174.8		103.0	169.1		108.7		170.6	C ₁
61.7		126.1		61.2	127.3		56		117.9	C ₂
98.0		162.4		90.1	156.2		91		152.9	C ₃
10.9		75.3		8.1	74.2		12.9		74.8	C ₄
13.6		78.0		13.6	79.7		6.4		68.3	C ₅
0		65.4		0	66.1		0		61.9	C ₆

experimental NMR contains water.

^a Exp. = Experimental.

^b Δ = The chemical shift relative to the most shielded H₆ of ¹H-NMR and C₆ of ¹³C-NMR spectra DMSO used in

^c Cal. = Calculated.

¹³C-NMR: Experimental carbon chemical shifts of **1** were obtained at C₁=170.6, C₃=152.9, C₂=117.9, C₄=74.8, C₅=68.3, C₆=61.9 in DMSO (Table 4). The calculated chemical shifts were found at C₁=169.1, C₃=156.2, C₂=127.3, C₅=79.7, C₄=74.8, C₆=66.1 in the gas phase. The calculated chemical shifts of carbons were observed at C₁=174.8, C₃=162.4, C₂=126.1, C₅=78.0, C₄=75.3, C₆=65.4 in water phase.

4.3.2 Calculated NMR spectrum of tautomers 2-4

¹H-NMR. The calculated chemical shifts of tautomers of L-ascorbic acid in the gas phase are presented in Table 5. The most acidic hydrogen of tautomers of L-ascorbic acid is H₄ that appeared at 9.7, 11.1 and 3.9 ppm for isomers **2**, **3** and **4** respectively.

Table 5. Calculated ^1H -NMR and ^{13}C -NMR spectrums of compound 2, 3 and 4 at DFT levels of theory using B3LYP/6-311+G(2d,p) basis set in gas phase.

^1H -NMR [TMS B3LYP/6-311+G(2d,p) GIAO]						
$\Delta\delta$	(4)	$\Delta\delta$	(3)	$\Delta\delta^a$	(2)	Assignment
0.0	-0.8.	0.0	-0.5	0.0	-0.7	H ₀₆
2.4	1.6	4.6	4.1	2.7	2.0	H ₀₅
-	-	7.4	6.9	6.2	5.5	H ₃
4.7	3.9	11.6	11.1	10.4	9.7	H ₄
4.5	3.7	-	-	4.8	4.1	H ₅
4.0	3.2	4.4	3.9	3.8	3.1	H ₆
4.1	3.3	4.5	4.0	4.2	3.5	H ₆
3.9	3.1	4.0	3.5	-	-	H ₂
4.0	3.2	5.8	5.3	6.8	6.1	H _{0r}
^{13}C -NMR [TMS B3LYP/6-311+G(2d,p) GIAO]						
$\Delta\delta$	(4)	$\Delta\delta$	(3)	$\Delta\delta$	(2)	Assignment
129.9	197.9	118.6	185.3	107.0	176.4	C1
79.4	147.4	78.0	144.7	118.0	187.4	C2
150.2	218.2	105.2	171.9	108.0	177.4	C3
21.8	89.8	53.7	120.4	68.5	137.9	C4
9.7	77.7	103.0	169.7	10.2	79.6	C5
0	68.0	0	66.7	0	69.4	C6

^a $\Delta\delta$ The chemical shift relative to the most shielded proton and carbon.

^{13}C -NMR: The calculated carbon chemical shifts of **2** was observed at C₂=187.4, C₃=177.4, C₄=176.4, C₅=137.9, C₆=79.6, C₆=69.4. The calculated carbon chemical shifts of **3** for C₁ (corresponding to carboxylic acid group) was observed at 185.3 ppm. The chemical shifts of the C₂ and C₃ atoms of the enol form were observed at 144.7 and 171.9 ppm, respectively. The chemical shift of C₄, C₅ and C₆ atoms was calculated at 120.4, 169.7 and 66.7 ppm, respectively. The calculated carbon chemical shifts of **4** appeared at 197.9, 147.4, 268.2, 89.8, 77.7 and 68.0 ppm for C₁, C₂, C₃, C₄, C₅ and C₆, respectively.

CONCLUSION

The single-excitation configuration interaction TD-DFT calculations of molecular orbital geometry in the gas and water phase predicted that the visible absorption maxima of the L-ascorbic acid correspond to the electron transition from HOMO to LUMO which attributed to maximum amount of the f values with the highest % absorption. The λ for L-ascorbic acid was calculated 238 nm max in the gas phase. This value was increased to 248 nm in the water phase (the solvent effects).

The order of $\nu(\text{O-H})$ modes for L-ascorbic acid were obtained experimentally $\nu(\text{O}_2-\text{H}_1) > (\text{O}_6-\text{H}_{06}) > (\text{O}_2-\text{H}_2) > (\text{O}_5-\text{H}_{05})$. The calculated values are $\nu(\text{O}_6-\text{H}_{06}) > (\text{O}_2-\text{H}_2) > (\text{O}_5-\text{H}_{05}) > (\text{O}_3-\text{H}_3)$ in the gas phase and $\nu(\text{O}_3-\text{H}_3) > (\text{O}_5-\text{H}_{05}) > (\text{O}_6-\text{H}_{06}) > (\text{O}_2-\text{H}_2)$ in water phase. The two stretching modes for the $\text{O}_5\cdots\text{H}_3$ hydrogen were measured at 838 and 280 cm^{-1} for the compound of **3**.

The experimental (in DMSO) and theoretical calculation chemical shifts of L-ascorbic acid predicted proton H₁ the most deshielded chemical shift. The most acidic hydrogen of tautomers of L-ascorbic acid is H₁ that appeared at 9.7, 11.1 and 3.9 for the **2**, **3** and **4**, tautomers, respectively.

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