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# Disentangling the Puzzle of Hydrogen Bonding in Vitamin C

Isabel Peña,[†] Adam M. Daly,[†] Carlos Cabezas,[†] Santiago Mata,[†] Celina Bermúdez,[†] Amaya Niño,[†] Juan C. López,[†] Jens-Uwe Grabow,[‡] and José L. Alonso*[,†]

[†]Grupo de Espectroscopía Molecular (GEM), Edificio Quifima, Laboratorios de Espectroscopia y Bioespectroscopia, Parque Científico UVa, Universidad de Valladolid, 47005 Valladolid, Spain

[‡]Institut für Physikalische Chemie und Elektrochemie, Lehrgebiet A, Gottfried-Wilhelm-Leibniz-Universität Hannover, Callinstraße 3A, D-30167 Hannover, Germany

S *Supporting Information*

ABSTRACT: Fast-passage Fourier transform microwave spectroscopy in combination with a laser ablation source has been successfully applied to probe vitamin C (L-ascorbic acid) in the gas phase. Its ethyldiol side chain and two hydroxyl groups around the γ-lactone ring provide five internal rotation axes, enabling vitamin C to assume a wide variety of nonplanar 3D cooperative hydrogen bond networks that can also include the keto and ether functions. The rotational constants extracted from the analysis of the spectrum unequivocally identify the existence of three dominant conformers stabilized by different intramolecular hydrogen bonding motifs forming five-, six-, or seven-membered rings.
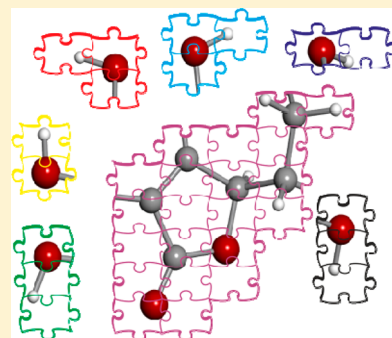
SECTION: Spectroscopy, Photochemistry, and Excited States

L-Ascorbic acid, known as vitamin C, has a central role in the development of molecular biochemistry and pharmacology. It was the first commercially synthesized vitamin, and since its first chemical isolation,[1] the study of this important molecule has been shown to provide insight into an array of biological functions[2−6] as well as the well-known antioxidant properties.[7,8] Vitamin C is a γ-lactone ring that contains two hydroxyl groups and an ethyldiol side chain. Their four hydroxyl groups can act simultaneously as proton donor or acceptor while ketone and ether groups act as proton acceptors. Thus, up to 11 intramolecular hydrogen bonds shown schematically in Figure 1 can be established within the molecule with three different types of hydrogen bonding: O–H···O=C (1), O–H···O (2,3), and O–H···O–H (4–11). (See Figure 1 for the labeling of the hydrogen bonds.) This hydrogen bonding puzzle can potentially stabilize a great number of low-energy conformers. High-level ab initio computations carried out in our laboratory give rise to up to 17 conformers within 900 cm^{-1} (see Table S1 of the Supporting Information). Those below 700 cm^{-1} are collected in the graphic in Table 1. A maximum of four intramolecular hydrogen bonds can be established in some of the predicted conformers that are labeled by numbers in parentheses indicating the hydrogen bonds. A suffix letter is added to distinguish conformers with the same hydrogen bonding network (see Table 1).

Owing to its key importance as a relevant biomolecule, vitamin C has been the subject of several structural studies in condensed phases. First results come from X-ray and neutron diffraction data.[9,10] Later studies including NMR,[11,12] IR,[13] fluorescence,[14] and ESR[15−17] have also been reported. Different

conformers have been proposed on the basis of the proton coupling constants from the NMR studies,[11,12] attributed to different configurations of the side chain. Despite these extensive efforts, there is still no conclusive experimental information on vitamin C revealing the spatial nature of its intramolecular hydrogen bonding that governs the molecule's five degrees of conformational freedom. An experimental study of the molecule under isolated conditions in the gas phase free from the bulk effects is required to disentangle the hydrogen bonding puzzle in vitamin C and to unveil its conformational behavior. To date, no gas-phase experiments of vitamin C have been reported, probably due to its high melting point (190 °C) and the associated low vapor pressure. In fact, gas-phase experiments of vitamins have been limited to photoelectron spectra of vitamins A[18] and D.[19]

Fourier transform microwave spectroscopy carried out in a supersonic expansion is ideally suited to discern among subtle changes in structure due to the high resolution and the capability to distinguish between different conformers in the spectra. In the past few years, the combination of this technique with laser ablation methods, LA-MB-FTMW,[20] has been demonstrated to be singularly successful in the definitive assignment of low-energy conformers of natural amino acids,[21] nucleic acid bases,[22] and neurotransmitters.[23] Two conformers of aspirin have been revealed by this technique.[24] Recently, a
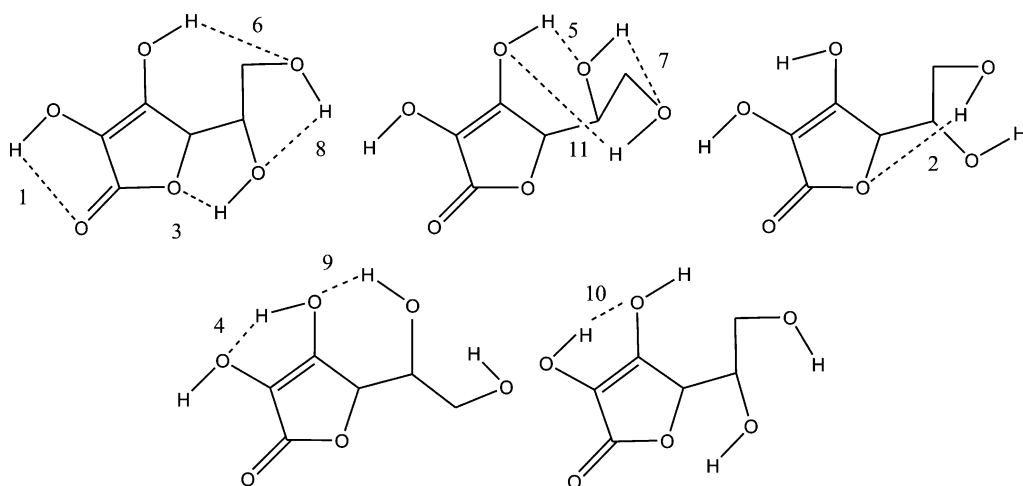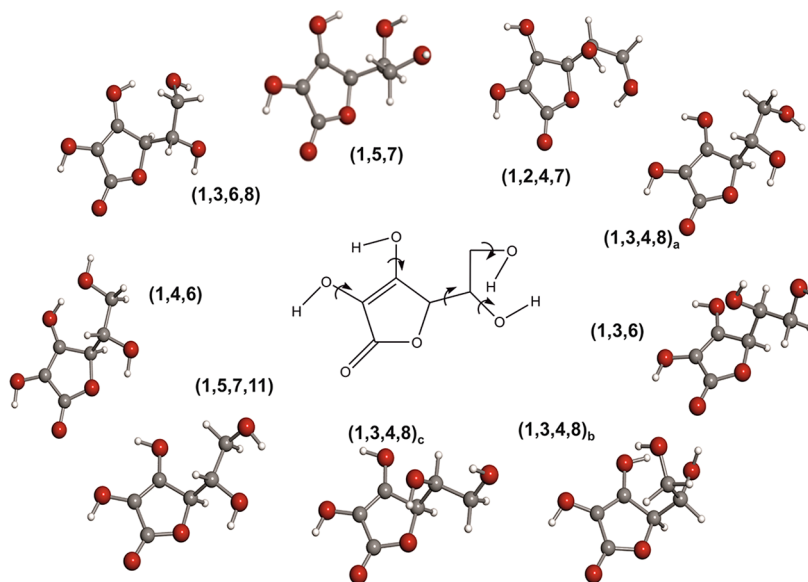
Figure 1. Different backbone arrangements showing a total of 11 plausible intramolecular hydrogen bonds of vitamin C.

Table 1. Calculated Spectroscopic Parameters and Ab Initio Energies at the MP2/6-311++G(d,p) Level of Theory for the Lowest-Energy Conformers of Vitamin C (below 700 cm^{-1}) Which Are Shown in the Graphic

	A^a	B	C	$|\mu_a|$	$|\mu_b|$	$|\mu_c|$	ΔE^b	ΔE^c	ΔG^d
(1,3,6,8)	1558	721	528	4.0	0.4	0.1	0	93	233
(1,5,7)	1472	682	578	4.1	0.2	1.1	55	37	53
(1,2,4,7)	1447	772	582	0.4	3.3	2.0	56	0	0
(1,3,4,8)$_a$	1621	666	504	0.3	2.3	1.2	302	228	177
(1,3,6)	1475	731	510	5.6	1.3	0.5	373	367	394
(1,3,4,8)$_b$	1617	620	481	1.1	2.3	1.0	575	449	378
(1,3,4,8)$_c$	1653	619	512	1.4	2.3	0.7	621	498	422
(1,5,7,11)	1425	775	681	2.6	0.2	0.6	647	665	755
(1,4,6)	1605	646	529	2.5	1.9	1.4	675	498	406

^{a}A, B, and C represent the rotational constants (in MHz); μ_a, μ_b, and μ_c are the absolute values of electric dipole moment components (in D). ^{b}MP2/6-311++G(d,p) electronic energies (in cm^{-1}). ^{c}Electronic energies corrected to zero-point vibrational energies calculated at the same level (in cm^{-1}). ^{d}Gibbs energies calculated at 298 K at the MP2/6-311++G(d,p) level of theory (in cm^{-1}).

new approach of high-resolution rotational spectroscopy that provides a sensitive method for broadband detection (CP-FTMW)[25] has been introduced, increasing the speed of the spectral acquisition and making more efficient the search of different coexisting species. We have combined a CP-FTMW spectrometer with a laser ablation source to investigate the rotational spectrum of nicotinic acid.[26]

In the present study on vitamin C, we have assembled a fast passage Fourier transform microwave spectroscopy called IMPACT (in-phase/quadrature-phase-modulation passage-acquired-coherence technique), used in the study of nicotine[27] with a laser ablation source trying to overcome the vaporization difficulties of vitamin C. The broadband microwave spectrometer, described in detail elsewhere,[27] has been modified,
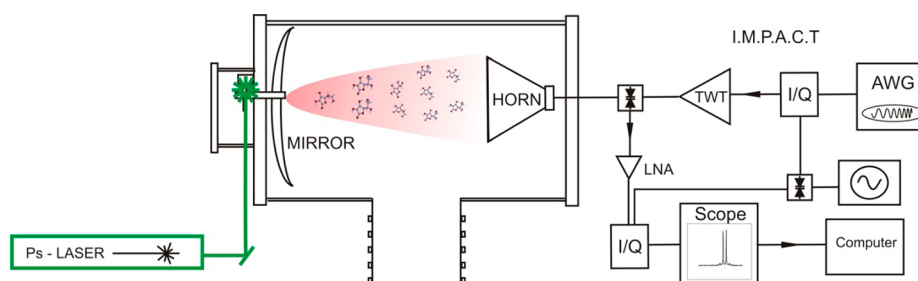
Figure 2. Fast passage Fourier transform microwave spectrometer with laser ablation source. (For more details, see the Supporting Information.)

replacing one of the horns of the original assembly by a concave aluminum mirror that supports the ablation nozzle (see Figure 2). Hence, a single horn is used for both polarization and detection events, which offer the well-known sub-Doppler resolution of a coaxial arrangement. Solid samples of vitamin C (mp ~190 °C) were ground and mixed with a minimum amount of a commercial binder, and the mixture was pressed into cylindrical rods that were placed into our ablation nozzle and vaporized using the third harmonic (355 nm) of a ps laser. Broadband surveys were carried out from 6 to 10 GHz in 1 GHz sections (see Figure 3). Two sets of R-branch μ_a-type
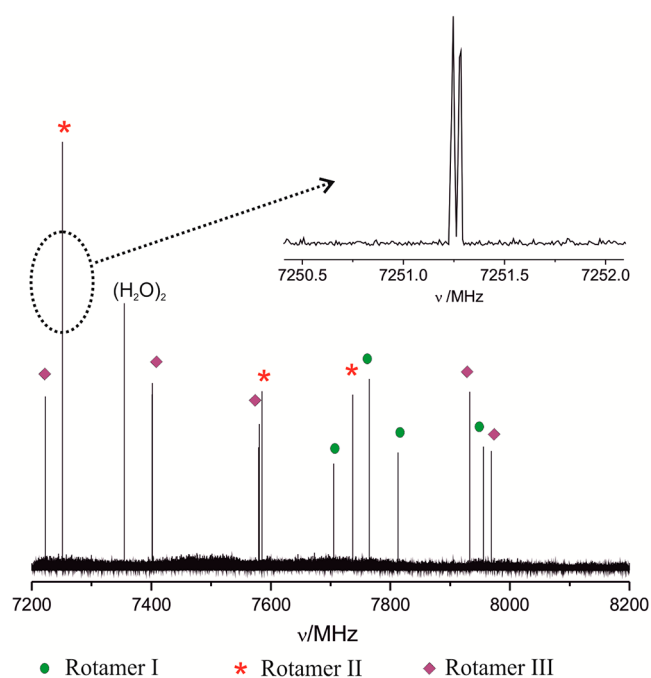

Figure 3. Section of the broadband spectrum of laser ablated vitamin C obtained from signal average of 9000 cycles at 2 Hz of repetition rate. The inset shows the $6_{0,6} \leftarrow 5_{0,5}$ rotational transition of rotamer II with sub-Doppler resolution.

transitions, according to a near prolate asymmetric top, were quickly identified as corresponding to two different rotamers labeled as I and II. Preliminary sets of rotational constants were obtained and used to predict and measure new μ_a-type lines. In addition, μ_b- and μ_c-type transitions were predicted for both rotamers, but only μ_c-type transitions for rotamer II could be detected. A third rotamer labeled III was revealed with a characteristic μ_b-type R-branch pattern; μ_c-type transitions were also measured, but no μ_a-type transitions could be detected for this rotamer. New measurements at lower frequencies (from 3
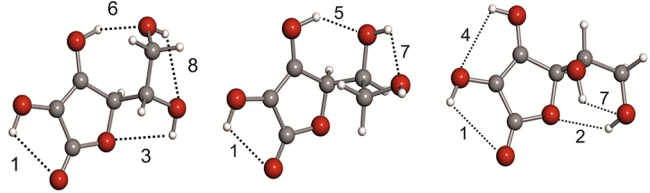
to 6 GHz) were subsequently done using our conventional LA-MB-FTMW spectrometer[20] corroborating the rotational assignments. The spectra of the three observed rotamers were analyzed[28] using the semirigid rotor Hamiltonian of Watson in the A reduction and the I^r representation to give the sets of rotational constants collected in Table 2. The measured transitions are given in Tables S2–S4 of the Supporting Information.

The first piece of evidence of the identification of the rotamers is the comparison of the experimental and predicted values of the rotational constants and the dipole moments for the lower energy conformers. We took into consideration the nine conformers below ~700 cm^{-1} of Table 1. The predicted values presented in Table 1 (see Table S1 of the Supporting Information for the complete listing) were generated by performing a series of ab initio geometry optimizations[29] using MP2 perturbation theory with a basis set including polarization functions on the heavy atoms (MP2/6-311+ +G(d,p)). The calculated values of the rotational constants exhibit a strong correlation with the side-chain conformation but only vary slightly with the hydroxyl group orientations. This is not too surprising as significant changes in the mass-distribution may occur for different side-chain configurations, whereas this would not happen by simply moving around a hydrogen atom.

The second step to confirm the identification of the observed rotamers is supplied by the magnitude of the electric dipole moment components along the principal inertial axes (μ_a, μ_b, μ_c). These tend to vary substantially when the orientation of any OH group is changed. Because of the highly directional nature of the free electron pairs at the hydroxyl group oxygen atom as the most polar part of the molecule, its orientation can dramatically impact the line strength of the a-, b-, and c-type rotational spectra to be observed. Although not explicitly measured, their magnitude could be approximately determined from the dependence of the intensity of the corresponding transitions upon the excitation power. The study of this dependence has been carried out from the LA-MB-FTMW experiments, where the microwave power needed for an optimal polarization of individual transitions is linearly correlated with the values of the electric dipole moment components.

Taking into account all of the above, the rotational constants of rotamer I in Table 2 are consistent only with those obtained for the global minimum conformer stabilized by four intramolecular hydrogen bonds (1,3,6,8). Rotamer I presents a strong μ_a-type spectrum, and the absence of b- and c-type transitions is consistent with the near-zero dipole moment values predicted for this conformer. There is an intramolecular hydrogen bond network (ring)−O−H··[6]··O−H··[8]··O−H··[3]··O-(ring) involving a hydroxyl group of the ring, the two hydroxyl

Table 2. Experimental and Predicted Rotational Parameters for the Three Observed Conformers of Vitamin C

	experimental	predicted[a]	experimental	predicted	experimental	predicted
	I	(1,3,6,8)	II	(1,5,7)	III	(1,2,4,7)
A^b	1562.4617(45)[c]	1558	1472.72595(37)	1472	1455.71362(17)	1447
B	715.14780(18)	721	678.05674(16)	682	760.47964(15)	772
C	524.25945(21)	528	575.00760(20)	578	575.43415(18)	582
Δ_J	0.0103(19)		0.0431(20)		0.0348(24)	
μ_a	Y[d]	4.0	Y	4.1	N	0.4
μ_b	N	0.4	N	0.2	Y	3.3
μ_c	N	0.1	Y	1.1	Y	2.0
σ	3.9		4.3		2.9	
N	28		43		34	

[a]Ab initio calculations performed at the MP2/6-311++G(d,p) level of theory. [b]A, B, and C are the rotational constants in MHz; μ_a, μ_b, and μ_c are the electric dipole moment components in D; Δ_J is the distortion constant in kHz; σ is the rms deviation of the fit in kHz; and N is the number of transitions. [c]Standard error in parentheses in units of the last digit. [d]"Yes" or "No" to observation of a-, b-, and c-type transitions for each structure.

groups of the side chain, and the ether group of the ring. The two hydroxyl groups on the side chain act as both hydrogen donor and acceptor. Rotamer II is only compatible with conformer (1,5,7) on the basis of values of rotational constants and the observation of strong μ_a and weak μ_c-type spectra in accordance with the predicted values of the dipole moment components of this conformer. The conformer (1,5,7) is stabilized by a (ring)−O-H··1··O═C intramolecular hydrogen bond and the hydrogen bond network (ring)−O-H··5··O−H··7··O−H connecting the hydroxyl group of the ring and the two hydroxyl groups of the side chain. The hydroxyl group located on the α-carbon of the side chain involved in both five and seven hydrogen bonds is the unique one acting as donor and acceptor groups. Rotamer III has been identified as a conformer (1,2,4,7) that is predicted to have a strong μ_b-type spectrum and is consistent with the observed spectrum. This conformer presents the three types of intramolecular hydrogen bonds in two different networks: (ring)−O-H··4··O−H··1··O═C and O−H··7··O−H··2··O(ring) involving hydroxyl, ketone and ether groups. The post-expansion abundances of the detected conformers have been evaluated by relative intensity measurements of selected transitions. The estimated ratios point out that the three conformers have a similar stability, in agreement with the relative energies predicted ab initio.

In conclusion, we have observed and identified for the first time three dominant conformers of vitamin C. These conformers exist in the gas phase as a result of hydrogen bonding networks of three different types of cooperative contacts: O−H····O−H, O−H····O(ring) and O−H····O═C, closing five-, six-, or seven-membered rings. Such arrangement of functional groups into hydrogen bond networks lead to a phenomenon known as cooperativity,[30−33] under which the strength of each individual hydrogen bond is notably increased. It is noticeable that the hydrogen bond 1, established between the ketone and one of the hydroxyl groups located on the ring, is highly stabilizing and is present in all conformers. Conformers without hydrogen bond type 1 are predicted to be at least 700 cm^{-1} above the global minimum.

Disentangling the pieces of this conformational puzzle demonstrates the discriminating power that the combination of rotational data with ab initio quantum chemical calculations delivers in the structural analysis of flexible biomolecules in the gas phase.

■ ASSOCIATED CONTENT

⑤ Supporting Information

Tables of molecular properties predicted ab initio for all conformers within 900 cm^{-1}; measured transitions and Cartesian coordinates for the three observed conformers of vitamin C; supplementary figures; LA-MB-FTMW spectra; description of the fast passage Fourier transform microwave spectrometer; and complete ref 29. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: jlalonso@qf.uva.es.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

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