

# Rotational spectrum of phenylglycinol

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## a b s t r a c t

Solid samples of phenylglycinol were vaporized by laser ablation and investigated through rotational spectroscopy in a supersonic expansion using two different techniques: chirped pulse Fourier transform microwave spectroscopy and narrow band molecular beam Fourier transform microwave spectroscopy. One conformer, bearing an O–H···N and an N–H···O intramolecular hydrogen bonds, could be successfully identified by comparison of the experimental rotational and  $^{14}\text{N}$  nuclear quadrupole coupling constants with those predicted theoretically.

## 1. Introduction

Unraveling the conformational panorama of biologically active molecules is of great importance, since it dictates the nature of the intermolecular interactions, itself a subject of great importance in molecular recognition processes, neurotransmission, intracellular transport and drug–receptor interaction [1]. The intrinsic complexity of biological systems, and thus, the difficulty of understanding conformational behaviors in such systems, implies that a tiered study must be undertaken, starting with the simplest system possible, and gradually increasing its complexity, by introducing new types of interactions at pace. Thus, it is of utmost importance to proceed to a detailed characterization of the conformational properties of the molecule, in isolation conditions, free from any kind of effects that may arise from interaction with other molecules and so perturb the conformational equilibria of the molecule, matching those found in gas-phase.

Several technical implementations in microwave spectroscopy turned it into a powerful tool for probing biomolecules properties. Supersonic expansion environment provides the ideal medium for virtually isolating the stable conformers of a molecule, allowing it to be interrogated by a short burst of microwave radiation. Moreover, the use of laser ablation helped studying molecules that would be otherwise unable to be vaporized using traditional heating methods. Nowadays, broadband microwave spectroscopic techniques (chirped pulsed Fourier transform microwave spectroscopy – CP-FTMW) [2], coupled with laser ablation, are being used to rapidly acquire the rotational spectra of solid compounds [2–5] in wide frequency ranges, making the search of different coexisting species in

the supersonic jet more effective. On other hand, narrowband LA-MB-FTMW [6], provides the sub-Doppler resolution necessary to analyze the nuclear quadrupole coupling hyperfine structure of the observed species, which is a unique tool for the definitive ascription of the low-energy conformers [7–9].

Phenylglycinol (PG), also known as 2-amino-2-phenylethanol, is the basis for a family of neurokinin 1 antagonists [10,11], which have been used successfully in the prevention of nausea and vomiting associated with cancer chemotherapy [12]. Its structure can be derived from ethanolamine, by substituting a C $\alpha$  vicinal hydrogen for a phenyl group, or by decarboxylation of phenylglycine, an unnatural amino acid. In addition, PG is also a structural isomer of APE (2-amino-1-phenylethanol), the latter being the building block of the adrenergic and noradrenergic system. PG (Figure 1) is expected to exhibit different conformations thanks to the flexibility of its ethanolamine moiety; the four existing degrees of freedom in the side chain generate several low-energy conformers. In this work, focus shall be given toward the theoretical prediction of the conformational landscape of phenylglycinol, followed by the experimental determination of the conformational panorama by identifying the most stable conformers present in gas-phase, and disclosing the intramolecular interactions contributing toward the stabilization of said conformers. To the best of our knowledge, only works on the amino alcohols APE [13], threoninol [14] and serinol [15] have been reported.

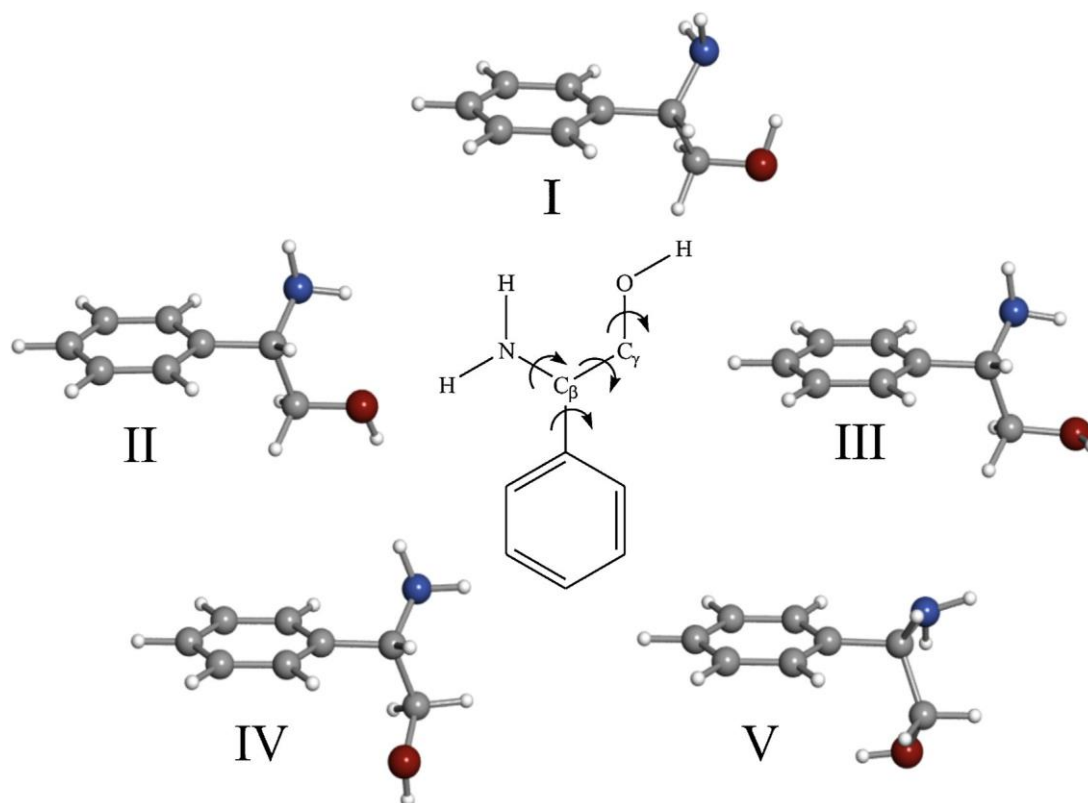
## 2. Methods

### 2.1. Experimental

The rotational spectrum of PG was obtained using two different Fourier transform microwave techniques with a laser ablation

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**Figure 1.** The possible degrees of freedom of the structure of PG, and the low-energy plausible conformers of PG, calculated at MP2/6-311++G(d,p).

source built in our laboratory [2,6]. In both experiments, PG (98%, m.p. = 76 ° C) samples were prepared by mixing the powder of the solid with a commercial binder. The mixture was pressed to form cylindrical rods, which were placed in a laser ablation nozzle to be vaporized using the third (355 nm) harmonic (12 mJ per pulse) of a 20 ps Nd:YAG laser. Afterwards, PG molecules were seeded in the carrier gas, Ne at backing pressure of 15 bar, and expanded adiabatically into the spectrometer cavity.

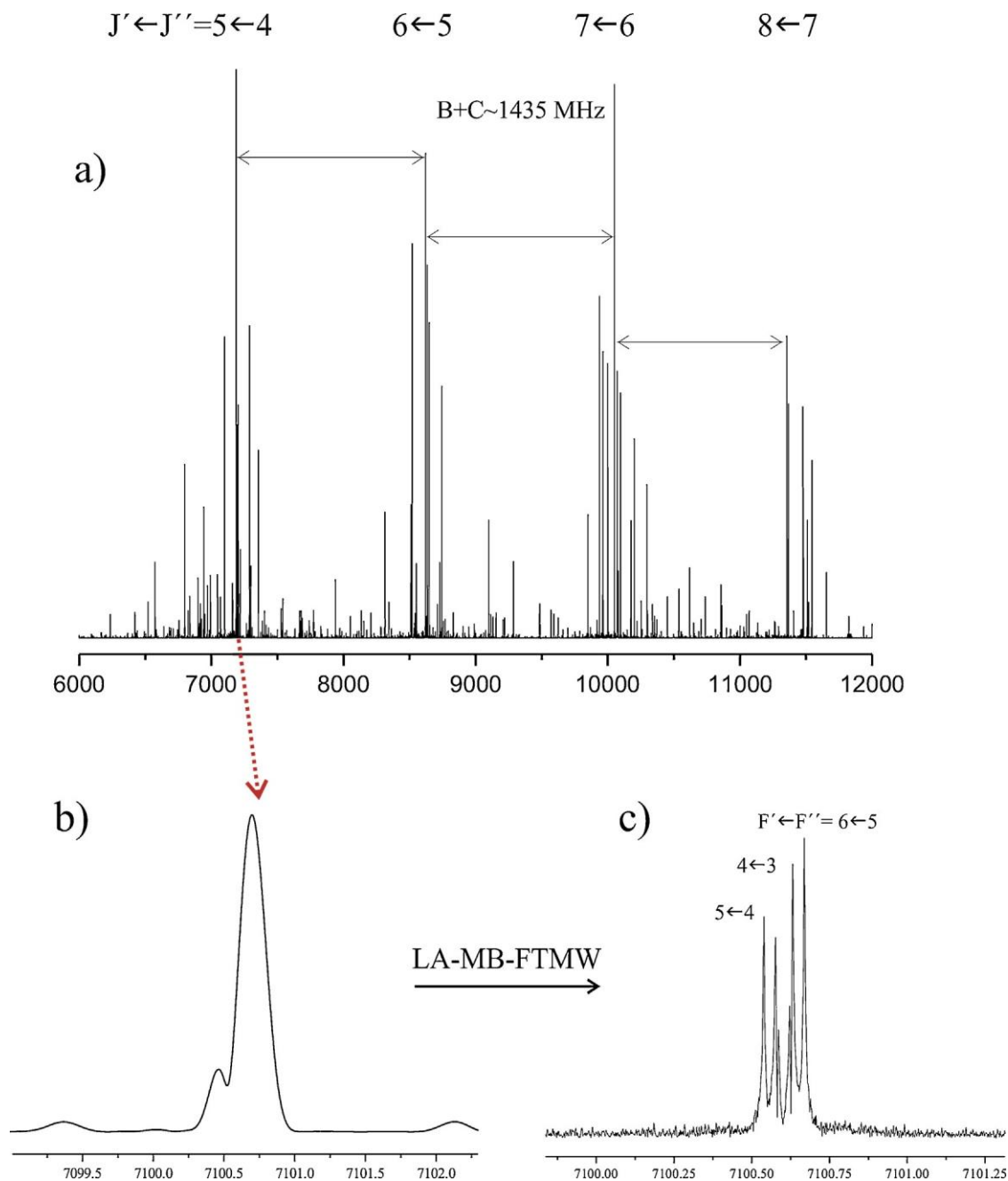
A chirped pulse Fourier-transform microwave (CP-FTMW) spectrometer [2] equipped with laser ablation vaporization system was first employed to sample swiftly the rotational spectra between 6.0 and 12.0 GHz of the different conformers present in the gas-phase mixture. The configuration of our laser ablation chirped pulse Fourier transform microwave spectrometer employed in this investigation, where the conventional microwave horns were replaced by the parabolic reflector system, has been previously presented [4]. An arbitrary waveform generator creates a chirped pulse, which is upconverted by a phase-locked dielectric resonator oscillator in a broadband mixer and amplified by a 300 W traveling wave tube amplifier. The excitation pulse emerged from a horn antenna to polarize the molecules arising from the laser ablation nozzle located at the center of one of the parabolic reflectors. The amplified pulse is broadcasted into the vacuum chamber through one microwave horn, interacting with the vaporized molecules in the pulsed jet. A second ridge horn antenna was used to detect the free induction decay signal (FID), which was finally amplified and digitized on a fast oscilloscope. The broadband frequency domain spectrum with 30,000 cycles is shown in Figure 2.

The LA-MB-FTMW spectrometer [6], covering the frequency range of 4–10 GHz, was used to record the PG spectrum with the necessary resolution to analyze the hyperfine structure due to the presence of a  $^{14}\text{N}$  nucleus. The optimal conditions to polarize the molecules in the jet correspond to molecular pulses of about 1.1 ms,

followed by MW polarization pulses of 0.3 ms duration with powers of 1–40 mW. The microwave transient FID was recorded for 100 J.s in the time domain at 40–100 ns sample intervals, and Fourier-transformed to the frequency domain. Due to the collinear disposition between the supersonic jet and the microwave resonator axis, all emission signals appeared to be split into Doppler doublets (Figure 2c). The arithmetic mean of the doublets was taken as the rest frequency. The estimated accuracy of the frequency measurements is greater than 3 kHz. From 50 to 250 averages were phase-coherently coadded to achieve reasonable signal-to-noise ratios.

## 2.2. Theoretical modeling

A general panorama of the conformational landscape of PG can be obtained by performing theoretical predictions, in order to ascertain the most stable conformers on the potential energy surface – of which only the low-lying energy conformers will be sufficiently populated in the supersonic jet lead to into observation in the rotational spectrum. Starting geometries for ab initio calculations are initially selected by considering all possible rotations around single bonds (see Figure 1) and identifying plausible intramolecular hydrogen bonds. Rotation around the  $\text{C}_\beta\text{--C}_\gamma$  bond produced three unstrained extended geometries, as seen in Figure 1. Further plausible structures can be generated from these three geometries by rotating  $\text{C}_\beta\text{--N}$  and  $\text{C}_\gamma\text{--O}$  bonds again considering all the possible intramolecular hydrogen bonds. In order to predict the most stable structures, a series of structural optimizations were conducted on the starting configurations using the Gaussian suite of programs [16]. Full geometry optimizations using second-order Møller–Plesset perturbation theory (MP2) in the frozen core approximation and Pople’s 6-311++G(d,p) basis set were carried out. Finally, the five conformers depicted in Figure 1



**Figure 2.** (a) The CP-FTMW spectrum of PG (30,000 signal averages); (b) the  $5_{05} \leftarrow 4_{04}$  rotational transition in detail for the observed conformer arising from the chirped spectrum; (c) the  $5_{05} \leftarrow 4_{04}$  rotational transition using the LA-MB-FTMW spectrometer, showing the  $^{14}\text{N}$  quadrupole hyperfine structure. Since the molecular beam and the microwave radiation travel parallel to each other, each rotational transition appears as a doublet due to the Doppler effect.

were predicted to be below  $1000\text{ cm}^{-1}$ . All these predicted conformers were confirmed to be local minima in the potential energy surface by checking the non-existence of negative values in their Hessian matrix. The predicted values of the rotational constants and nuclear quadrupole coupling constants are collected in Table 1.

### 3. Results and discussion

The recorded broadband spectrum, shown in Figure 2, allowed identification of very intense  $J_a$ -type R-branch progressions of the pair of  $J_a$ -R-branch transitions  $J + 1_{0,J+1} \leftarrow J_{0,J}$  and  $J + 1_{1,J+1} \leftarrow J_{1,J}$  (with  $J$  ranging from 3 to 8), belonging to a single one rotamer, with  $J_b$ - and  $J_c$ -type transitions being subsequently predicted and measured. No lines attributable to other species were found in the

spectrum. PG presents one  $^{14}\text{N}$  nucleus with a nonzero quadrupole moment which interacts with the electric field gradient created at the site of the N nucleus by the rest of molecular charges. This interaction leads to the coupling between the  $^{14}\text{N}$  nuclear spin ( $I = 1$ ) with the overall angular momentum, giving rise to a hyperfine structure in the rotational spectrum. As shown in Figure 2b the resolution attained with the CP-FTMW technique is not sufficient to analyze this hyperfine structure. For this reason, only the center of frequencies of the hyperfine clusters were measured and fitted, leading to an initial set of rotational constants ( $A = 3087.3\text{ MHz}$ ;  $B = 738.4\text{ MHz}$ ;  $C = 700.9\text{ MHz}$ ). A first comparison of the experimental values with those predicted for the lowest energy conformers (Table 1), indicate that the observed rotamer is within the parameters predicted for conformers I, II or III although

**Table 1**  
Calculated spectroscopic parameters for the lowest energy conformers of PG.

	I	II	III	IV	V
A <sup>a</sup>	3085.2	3053.6	3019.7	2255.3	2459.5
B	735.7	757.9	756.9	936.0	918.0
C	700.9	700.2	699.2	804.3	838.1
x <sub>aa</sub>	-4.54	1.35	1.14	1.91	-0.41
x <sub>bb</sub>	2.27	-0.82	-0.84	1.59	-0.60
x <sub>cc</sub>	2.27	-0.53	-0.31	-3.49	1.01
i <sub>-a</sub>	-2.8	0.1	-0.6	1.0	0.3
i <sub>-b</sub>	0.9	-0.6	-1.7	0.3	-1.0
i <sub>-c</sub>	1.4	-0.7	1.3	-0.2	-0.7
◆E <sup>b</sup>	0	115	330	773	678
◆G <sup>c</sup>	0	116	368	674	707

<sup>a</sup> A, B and C are the rotational constants, in MHz; x<sub>aa</sub>, x<sub>bb</sub> and x<sub>cc</sub> are the <sup>14</sup>N nuclear quadrupole coupling constants, in MHz; i<sub>-a</sub>, i<sub>-b</sub> and i<sub>-c</sub> are the electric dipole moment components, in Debye.

<sup>b</sup> Relative energies respect to the global minimum calculated at MP2/6-311++G(d,p) level of theory, in cm<sup>-1</sup>.

<sup>c</sup> Gibbs energies calculated at 298 K at the MP2/6-311++G(d,p) level of theory in cm<sup>-1</sup>.

a conclusive identification cannot be achieved solely on the basis of the rotational constants.

In the next step of the investigation, PG was probed by utilizing the sub-Doppler resolution of our LA-MB-FTMW spectrometer. This made possible to resolve the hyperfine structure of the observed rotamer for several rotational transitions measured in the frequency range 4–10 GHz. Figure 2c shows the hyperfine structure of 5<sub>05</sub>–4<sub>04</sub> transition. The frequencies measured for the different quadrupole coupling components hyperfine structure are collected in Table S1 of the supplementary data. They were fitted [17] using a semirigid rotor Hamiltonian H<sub>R</sub><sup>(A)</sup> supplemented with a H<sub>Q</sub> term to account for the nuclear quadrupole coupling contribution [18]. The Hamiltonian was set up in the coupled basis set I + J = F and diagonalized in blocks of F [19]. The energy levels involved in each transition are thus labeled with the quantum numbers J, K<sub>-1</sub>, K<sub>+1</sub>, F. The experimentally determined rotational constants A, B, C and 11<sub>J</sub> and the nuclear quadrupole coupling constants x<sub>aa</sub>, x<sub>bb</sub>, and x<sub>cc</sub> for the observed species are given in Table 1.

The conformational assignment of the observed rotamer has been achieved by comparing the experimentally determined spectroscopic constants with those predicted ab initio in Table 1. As mentioned before, a conclusive identification cannot be reached using rotational constants values since the predicted values for all conformers are similar. But conformers showing slight geometrical changes can be unambiguously identified from the values of nuclear quadrupole coupling constants, since they are very sensitive to the chemical environment of the nitrogen atom (Table 1). Hence the different arrangements of the NH<sub>2</sub> group in each conformer markedly change the values of the constants x<sub>aa</sub>, x<sub>bb</sub> and x<sub>cc</sub> for the <sup>14</sup>N that can be used as a key to identify the observed rotamer. Thus, a final comparison between the experimental and

theoretical values for those constants clearly serves to discriminate between conformers I, II and III and allows the unequivocal identification of the observed rotamer as conformer I. This assignment is further confirmed attending to the predicted values of the dipole moments and the type of spectra observed since strong a-type and weaker b- and c-type transitions were measured.

No lines attributable to other low-energy conformers were found in the spectrum. Although conformer II is predicted only 115 cm<sup>-1</sup> above conformer I, its detection in the rotational spectrum does not seem feasible given the predicted low values of the dipole moment components. On the other hand, conformer III, which differs from conformer II solely in the orientation of the OH group in the side chain, may collisionally relax to conformer II and could be absent in the supersonic expansion.

The conformational behavior of PG can be understood in the context of the intramolecular forces acting within in each conformer. The experimental determination of the <sup>14</sup>N quadrupole coupling constants constitutes an exceptional tool that allows the establishment of the orientation of the –NH<sub>2</sub> group with respect to the molecular frame. Those constants can be used to deduce the nature of the intramolecular interactions in which this functional group is involved. As can be seen in figure of Table 2 conformer I is stabilized by intramolecular O–H···N and an N–H···π cooperative hydrogen bonds, which contribute to the overall stability of the structure.

An excellent agreement between the experimental and predicted spectroscopic constants of conformer I has been found. Scale factors ranging from 1.000 up to 1.004 brings the ab initio values of the rotational constants for conformer I nearly into coincidence with the experimental values for the observed species. Hence, we can infer that its actual geometry should be very close to that calculated ab initio (given in Table S2 of the Supplementary Data) and

**Table 2**  
Experimentally determined spectroscopic parameters of the detected rotamer of PG.

A <sup>a</sup>	3087.32319 (50) <sup>b</sup>	
B	738.35982 (15)	
C	700.88590 (16)	
◆J	0.0423 (37)	
x <sub>aa</sub>	-4.2633 (31)	
x <sub>bb</sub>	2.2186 (36)	
x <sub>cc</sub>	2.0447 (36)	
a	1.8	
N	40	

<sup>a</sup> A, B and C are the rotational constants, in MHz; ◆J is a quartic centrifugal distortion constant, in kHz; x<sub>aa</sub>, x<sub>bb</sub> and x<sub>cc</sub> are the <sup>14</sup>N nuclear quadrupole coupling constants, in MHz; a is the standard deviation, in kHz; N is the number of fitted transitions.

<sup>b</sup> Standard errors indicated in parentheses in units of the last digit.

can be taken as a good description of the actual structure of the PG conformers depicted in the figure of Table 2.

#### 4. Conclusions

The detection of one rotamer of neutral PG, and its posterior ascription to conformer I, provides a global picture of the behavior of an  $\alpha$ -amino-alcohol with an aromatic side chain. Conformer I is stabilized by an O–H $\cdots$ N bond, within the ethanolamine moiety, and one N–H $\cdots$ IT bond with the side chain. The present results deem both the CP-FTMW and the LA-MB-FTMW as excellent experimental platforms to face the challenge of investigating larger systems with an expected rich conformational behavior. This investigation contributes to an improved understanding of the role of intramolecular forces in the conformational preferences of amino alcohols.

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