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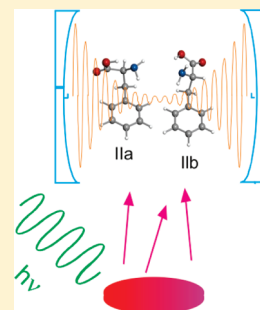
Jet-Cooled Rotational Spectrum of Laser-Ablated Phenylalanine

Cristóbal Pérez, Santiago Mata, Susana Blanco, Juan C. López, and José L. Alonso*

Grupo de Espectroscopia Molecular (GEM), Edificio Quifima, Área de Química-Física, Parque Tecnológico, Campus Miguel Delibes, Universidad de Valladolid, 47005 Valladolid, Spain

S Supporting Information

ABSTRACT: The rotational spectrum of neutral phenylalanine has been recorded for the first time using laser-ablation molecular-beam Fourier transform microwave spectroscopy (LA-MB-FTMW). Two conformers stabilized by conjugative $\text{O}-\text{H}\cdots\text{N}$ and $\text{N}-\text{H}\cdots\pi$ hydrogen bond interactions have been conclusively identified on the basis of experimental values of rotational and ^{14}N nuclear quadrupole coupling constants. The nonobservation of the rotational spectra of the other low-energy conformers has been attributed to the photofragmentation that takes place in the laser ablation process. Nuclear quadrupole coupling interactions have been used directly to determine the orientation of the amino group and to identify and experimentally characterize the $\text{N}-\text{H}\cdots\pi$ interactions.



INTRODUCTION

The identification of the different conformations that natural amino acids can adopt,¹ as well as the intramolecular interactions that stabilize them, is essential for the understanding of the protein function since the biological activity of these systems is controlled not only by the sequence of their building blocks but also by their conformation. The only way to quantify the factors that lead to the conformational stability of amino acids is to investigate them in the isolation conditions of the gas phase to observe the intrinsic molecular properties of these biochemical systems. With this purpose, supersonic jets combined with different vaporization methods and several spectroscopic techniques have been employed.^{1–3} Using electronic spectroscopy, phenylalanine,^{4–9} tyrosine,^{4,10} and tryptophan,^{11–13} bearing a chromophore aromatic ring, have been studied. Other proteinogenic amino acids, either with nonpolar^{14–17} or with polar^{1,18,19} side chains, as well as the iminoacid proline,²⁰ have been recently studied by using laser-ablation molecular-beam Fourier transform microwave spectroscopy (LA-MB-FTMW).^{1,21} With this technique, up to seven conformers in serine¹⁸ and threonine¹ and six conformers in cysteine¹⁹ have been identified.

The conformational landscape of phenylalanine (Phe) has been widely investigated.^{4–9,22–24} The six conformers shown in Table 1 were identified by Levy's⁴ and Simon's groups⁵ using laser-induced fluorescence (LIF)⁴ and hole burning UV–UV and ion dip IR–UV spectroscopy coupled with ab initio calculations.⁵ Later, Kim's group^{6–8} reported a revised assignment of the electronic spectrum by investigating the conformation-dependent ionization of Phe. A definitive identification of the conformers of Phe was done by Lee et al.⁹ based upon comparisons between the partially resolved ultraviolet band contours and that simulated by ab initio computations.

On this basis, we present here the first rotational study of Phe using LA-MB-FTMW spectroscopy.²¹ Two structures of the

related phenylglycine have been previously identified in the gas phase using this technique.²⁵ The spectroscopic information provided in this work could be relevant to check the existence of Phe in the interstellar medium (ISM)²⁶ that requires precise laboratory data.

EXPERIMENTAL DETAILS

The rotational spectrum of Phe was investigated by using an LA-MB-FTMW spectrometer,²¹ which works in the 5–18 GHz frequency region. In this experiment, neutral Phe molecules are vaporized by laser ablation of a solid sample (mp 270–275 °C) using the third harmonic (355 nm) of a pulsed picoseconds Nd:YAG laser (30 ps). Experiment using other laser wavelengths and large pulse widths (ns) lead to poorer results. The vaporized molecules are seeded in the carrier gas (Ne: 5 bar) and supersonically expanded into a Fabry–Pérot resonator where they are probed by Fourier transform microwave spectroscopy. This consists of the application of a continuous wave (CW) microwave radiation pulse (typ. 0.5 μs , <200 mW), in the operating frequency range of the spectrometer to cause the macroscopical polarization of the molecules in the beam. After excitation, molecular relaxation produces a transient microwave emission or free-induction-decay (FID). This is down converted to the radio frequency (RF) region, recorded in the time-domain, and Fourier transformed to yield the frequency-domain spectrum. Due to the collinear disposition between the supersonic jet and the microwave resonator axis, each line in the spectrum appeared as a Doppler doublet. The transition rest frequencies are

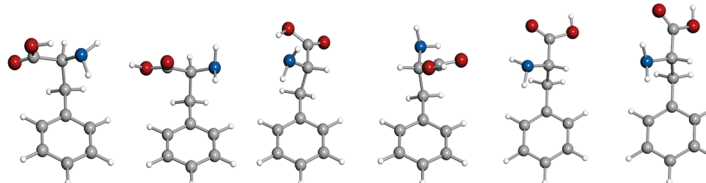
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Table 1. The Six Lower Energy Conformers of the L-Phenylalanine and Predicted Ab Initio Molecular Properties

	IIa	Ia	IIb	Ib	Ic	Id
A ^a / MHz	1657.6	1579.9	2451.2	1738.5	2416.2	2465.2
B / MHz	649.9	686.7	464.2	636.0	466.0	463.0
C / MHz	580.4	597.0	429.0	577.6	427.7	426.0
μ_a / D	2.5	0.4	5.3	0.6	1.9	1.2
μ_b / D	5.0	0.6	2.0	0.2	1.3	1.8
μ_c / D	0.60	1.0	2.2	1.4	1.1	0.8
χ_{aa} / MHz	0.28	1.54	-0.63	2.87	0.365	1.082
χ_{bb} / MHz	0.84	-2.32	-0.31	-4.67	2.719	1.428
χ_{cc} / MHz	-1.12	0.78	0.94	1.60	-3.085	-3.230
ΔE / cm ⁻¹	0	28	297	316	605	684



^a A, B, and C are the rotational constants. ^b μ_a , μ_b , and μ_c are the electric dipole moment components (absolute values quoted). ^c χ_{aa} , χ_{bb} , and χ_{cc} are the diagonal elements of the ¹⁴N nuclear quadrupole coupling tensor. ^d ΔE are relative energies calculated at the MP2/6-311++G(d,p) level of theory with zero-point energy correction using Gaussian 03.³¹

calculated as the arithmetic mean of the Doppler components. Different experiments, at the same frequency polarization, can be phase-coherently coadded, so a thousand cycles can be made for the measurements of very weak transitions. The estimated accuracy of the frequency measurements is greater than 3 kHz.

RESULTS AND DISCUSSION

Spectroscopic searches in the 4–10 GHz frequency range were directed to the identification of the conformers given in Table 1. In order to guide rotational assignments, we have extended previous ab initio calculations^{5,24} to predict the molecular properties of these conformers such as rotational constants, quadrupole coupling parameters and electric dipole moment components (see Table 1), which can be directly compared to the experimental ones in the interpretation of rotational spectra. All conformers were predicted to be near-prolate asymmetric tops. Initially, wide frequency scans with low polarization power were conducted to search for rotational transitions of conformers predicted to have a relatively large μ_a electric dipole moment component in order to detect the μ_a -type R-branch transitions, which show the characteristic patterns consisting of groups of lines separated approximately by $B + C$. The sets of R-branch lines corresponding to two different rotamers, initially labeled X and Y, were detected. These transitions were found to be rather weak and showed fully resolved ¹⁴N nuclear quadrupole coupling hyperfine structure (see Figure S1 of the Supporting Information), which confirms that the lines belong to Phe conformers. This hyperfine structure arises from the interaction of the electric quadrupole moment (eQ) of the ¹⁴N ($I = 1$) nucleus with the electric field gradient created at the site of the quadrupolar nucleus by the rest of the molecule. This interaction couples the ¹⁴N nuclear spin with the overall angular momentum, which results in a nuclear hyperfine structure in the rotational spectra. The associated spectroscopic parameters are the quadrupole coupling constants $\chi_{\alpha\beta}$ ($\alpha, \beta = a, b, c$), which are related to the electric field gradient elements $q_{\alpha\beta}$ by $\chi_{\alpha\beta} = eQq_{\alpha\beta}$. These constants are very sensitive to the electronic environment of the quadrupolar nucleus, in this case the amino group N atom,

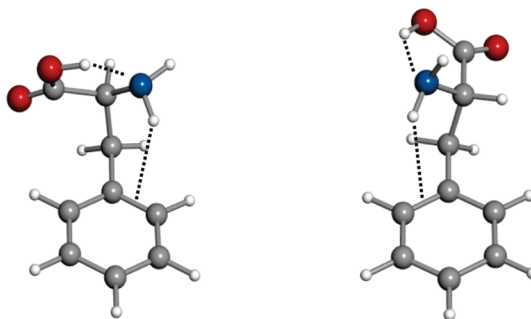
and to its orientation with respect to the principal inertial axes a , b , and c .

The spectra of the two observed rotamers were analyzed²⁷ using a semirigid rotor Hamiltonian of Watson in the A reduction and the I^r representation $H_R^{(A)}$,²⁸ supplemented with a term to take into account the quadrupole interaction H_Q ,²⁹ namely $H = H_R^{(A)} + H_Q$. The spectroscopic constants obtained from the initial fits allowed us to predict the μ_b - and μ_c -type spectra of these two rotamers. For rotamer X, only μ_b -type rotational transitions were found, while for rotamer Y sets of μ_b - and μ_c -type transitions were observed. In both cases, the new lines were found to be rather weak as found for the μ_a -type spectra. The low polarization powers used to observe them indicate that the electric dipole moment components, responsible for the corresponding selection rules, are large. The full sets of measured transitions were finally fitted to determine the rotational constants and diagonal elements of the quadrupole coupling tensor (χ_{ii} ; $i = a, b, c$) listed in Table 2. The observed transition frequencies for rotamers X and Y are respectively collected in Tables S1 and S2 of the Supporting Information.

Once we had the final set of rotational parameters for conformers X and Y, we proceeded to their identification by comparing the experimental observations with the theoretical predictions. The rotational constants of rotamer X (see Table 2) have values that can be compared to those predicted for species IIa, Ia, or Ib (see Table 1). If we consider the predicted quadrupole coupling constants of these three conformers, it is easy to see that only the values for conformer IIa are comparable, with minor discrepancies, to those observed for rotamer X. Furthermore, only conformer IIa is predicted to have values of μ_a and μ_b electric dipole moment components large enough to be observed with the low polarization powers used to measure the spectra. On this basis, rotamer X can be unequivocally assigned to conformer IIa. The set of rotational constants of rotamer Y (see Table 2), are consistent with those predicted for conformers IIb, Ic, and Id of Phe (see Table 1). However, if we compare the experimental values of the quadrupole coupling constants with the predicted ones, there is reasonable agreement only for conformer IIb. The matching between the large dipole moment

Table 2. Experimental Spectroscopic Constants for the Two Observed Rotamers of Phe

	IIa (X)		IIb (Y)	
	¹⁴ N	¹⁵ N	¹⁴ N	¹⁵ N
A / MHz	1666.0436(14) ^b	1646.7381(17)	2457.05490(48)	2425.69(30)
B / MHz	638.56314(12)	636.56611(19)	460.659722(79)	459.64825(32)
C / MHz	568.76843(15)	565.83617(20)	424.74604(13)	423.71892(25)
Δ _J / kHz	0.1002(16)	0.09660(19)	0.0171(12)	-
χ _{aa} / MHz	-0.283(16)	-	-0.777(22)	-
χ _{bb} / MHz	1.275(55)	-	0.0695(36)	-
χ _{cc} / MHz	-0.992(39)	-	0.7075(14)	-



^a A, B, and C are the rotational constants; Δ_J is a quartic centrifugal distortion constant; χ_{aa}, χ_{bb}, and χ_{cc} are the diagonal elements of the ¹⁴N nuclear quadrupole coupling tensor. ^b Standard error in parentheses in units of the last digit.

components predicted for this conformer and the low polarization powers required to observe the spectra further support the assignment of rotamer Y to conformer IIb.

Once we identified the observed rotamers, our following experimental efforts were focused to search for other conformers. We systematically performed wide frequency scans in the regions where the transition frequencies of the different low energy conformers were predicted. In each case we used different polarization powers to ensure optimal observation of the lines. We paid especial attention to the searches of the spectra of conformer Ia, which is predicted to be more stable than the observed conformer IIb. Despite these careful searches we fail to observe new conformers.

We acquired a ¹⁵N (*I* = 0) isotopically enriched sample of Phe, where the associated rotational transitions do not show quadrupole splittings, thus increasing the intensity of spectra. The rotational constants of the IIa and IIb conformers of ¹⁵N-Phe were first calculated from the predicted structures. Using these constants, the spectra of conformers IIa and IIb were observed at frequencies very close to the predicted ones. The spectra were analyzed using the same semirigid rotor Hamiltonian²⁸ $H_R^{(A)}$ used for the parent species. The obtained rotational constants are listed in Table 2, and the measured transitions are collected in Tables S3 and S4 of the Supporting Information. Using this ¹⁵N enriched sample, we proceed to repeat the same kind of searches carried for the parent employing different microwave polarization power conditions to search for either μ_a, μ_b, or μ_c-type transitions. Unfortunately no additional lines were observed in the spectrum of ¹⁵N-Phe.

The low intensity of the observed spectra of Phe and the nonobservation of the conformers detected with other techniques^{4–9,22–24} deserve some explanation. This is especially true in the case of conformer Ia, which is predicted to be more stable than the observed conformer IIb. In a usual supersonic jet experiment based on the expansion of gas mixtures in thermal equilibrium or when heating techniques are used to vaporize molecules, the relative population of conformers in the jet can be directly related to the preexpansion equilibrium distribution.

In laser ablation experiments on amino acids, similar results^{14a,30} have led to the assumption that conformers in the ablation plume are brought close to the equilibrium ratio at the temperature of the carrier gas flow previous to the expansion. In these conditions, the hypothetical relative intensities of the spectra of the different conformers can be estimated from the ab initio energy data and electric dipole moment components by assuming that in the supersonic expansion all the conformers are cooled down to their respective ground vibrational states. The estimation of the relative intensities of Phe conformers in this way indicates that at least the spectra of conformers Ia and IIb should be observable. Their nonobservation could indicate that these conformers, if present in the supersonic expansion, have proportions lower than those calculated from this model. In the case of Phe, an aromatic amino acid, photofragmentation can play an important role in the laser ablation vaporization process. To probe this fact, the ablation process was monitored using a time-of-flight mass spectrometer (TOF-MS) coupled with the same laser ablation nozzle used in our LA-MB-FTMW experiment. We tried to keep the same experimental conditions, but we could observe that the laser energy conditions needed to observe a good signal in the MW experiment and in TOF-MS are not the same. In the microwave experiment, the laser energy should be much higher than that needed for mass detection. As shown in Figure S2 of the Supporting Information, a large amount of Phe fragments were observed, together with the amino acid monomer, indicating a large degree of degradation of Phe in the vaporization process. The degree of degradation was found to decrease when lowering the laser energy. The laser energy needed to observe the MW spectra implies a high degree of fragmentation, and this would explain the weakness of the spectra observed. On the other hand, the nonobservation of all possible conformers could be related to a different fragmentation rate for the different conformers. In this context, Kim and co-workers,⁶ from their measurements of the ionization energies (IEs) of the low-energy conformers of Phe, affirm that the observed conformers IIa and IIb have higher IEs than the other conformers. If the nonobserved conformers are

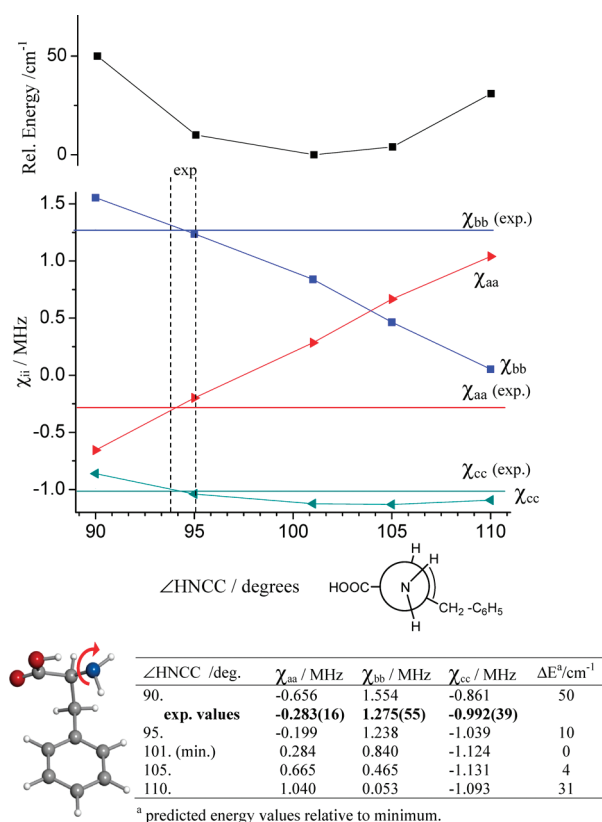


Figure 1. Variation of the relative energies and quadrupole coupling constants calculated at the MP2/6-311++G(d,p) basis set level with the dihedral angle HNCC for the conformer IIa of Phe.

ionized in the laser ablation process, these would be not present in the supersonic jet.

As in phenylglycine,²⁵ the small discrepancy between the experimental and predicted quadrupole coupling constants can be explained by taking into account that a little variation in the orientation in the amino group gives rise to a completely different set of quadrupole constants.¹⁴ As an example, we show in Figure 1 the values of the quadrupole coupling constants at different orientations of the $-\text{NH}_2$ group calculated by rotating it around the C–N axis. The results indicate that a good agreement between observed and calculated constants is achieved when the amino group is rotated only 6° from the predicted ab initio value of the dihedral $\angle\text{HNCC}$, without significantly changing the rotational constants. The electronic energies compiled in Figure 1 show a rather flat potential energy surface for the rotation around the C–N axis in the vicinity of the minimum. The modified orientation of the amino group shows an arrangement more favorable to the establishment of a $\text{N}-\text{H}\cdots\pi$ interaction than that for the optimized energy.

In conclusion, two conformers of Phe have been characterized in the gas phase through the analysis of its rotational spectrum. Both conformers exhibit a trans configuration in the COOH group, being stabilized by a $\text{O}-\text{H}\cdots\text{N}$ and $\text{N}-\text{H}\cdots\pi$ intramolecular hydrogen bonds. Nuclear quadrupole coupling interactions have been shown to be a useful tool to identify the observed conformers. These constants are very sensible to the orientation of the amino group, which, for the observed conformers, are consistent with the existence of $\text{N}-\text{H}\cdots\pi$ interactions stabilizing them.

■ ASSOCIATED CONTENT

S Supporting Information. Tables of the rotational transitions measured for conformers IIa and IIb of Phe for ^{14}N and ^{15}N isotopologues (Four Tables S1–S4). Tables with the Cartesian coordinates for the lowest energy conformers of Phe from ab initio MP2/6-311++G(d,p) calculations (Table S5). Figure of the $6_{0,6} \leftarrow 5_{0,5}$ rotational transition for the two detected conformers of the parent ^{14}N species of Phe (Figure S1). Mass spectrum of Phe vaporized through the laser ablation technique (Figure S2). 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. Phone: +34 9831866348. Fax: +34 983186349.

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