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Elucidating the Multiple Structures of Pipecolic Acid by Rotational Spectroscopy

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Abstract

The complex conformational space of the non-proteinogenic cyclic amino acid pipecolic acid has been explored in the gas phase for the first time. Solid pipecolic acid samples were vaporized by laser ablation and expanded in a supersonic jet where the rotational spectral signatures owing to nine different conformers were observed by Fourier transform microwave spectroscopy. All species were identified by comparison of the experimental rotational and nuclear quadrupole coupling constants with those predicted theoretically. Observation of type-III conformers, leading difference compared with the conformational behavior of the analog amino acid proline, has been interpreted by an increment in steric hindrance when increasing the number of carbons present in the ring.

Introduction

In biological molecules, flexible degrees of freedom are generally a synonym for possessing a multitude of conformational minima. Interestingly, it is not unusual for biological molecules to adopt but a few preferential conformations, despite the plethora of possible structures. Reasoning on such molecular preferences implies knowledge on both the intra- and intermolecular forces involved, which play an essential role in small, biologically active molecules with functions that are mediated via receptor site docking or hydrogen-bond binding. Indeed, one primary goal would be the full comprehension of the conformational preferences, as well as following biological functions, of molecules in the physiological environment - but the difficulty in assessing solvent effects turns this into a daunting task. Gas phase studies come as an ideal starting point, given that, in the gas phase, molecules can be considered to be virtually isolated - thus being freed from solvent effects - and effectively allowing assessment of intramolecular interactions solely. Furthermore, gas-phase data can be easily contrasted with theoretical models and used to refine the latter. An excellent motivation for this kind of work has been written by Weinkauff and co-workers.¹

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Conflicts of interest

There are no conflicts to declare.

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A plethora of spectroscopic techniques have been used, either in gas phase or in conditions mimicking isolation conditions, to survey the physical nature - with special emphasis on both the structure as well as the conformational topology of biomolecules, among amino acids,² peptides,³ sugars^{4–7} and neurotransmitters.^{8–10} Among the techniques applied in the gas phase to investigate the structures of biomolecules, rotational spectroscopy provides a means to unambiguously discriminate individual conformations of a flexible molecule, meeting the expectations previously set.¹¹ The combination of Fourier transform microwave (FTMW) spectroscopy with laser ablation (LA) techniques at GEM allowed characterization of conformational preferences of tenths of biomolecules among amino acids,¹¹ peptides,¹² sugars^{13,14} or neurotransmitters.¹⁵ Previous investigations of amino acids include, among others, over 25 proteinogenic and non-proteinogenic α -amino acids.¹ From these studies, it can be inferred that α -amino acids with a non-polar side chain (alanine,¹⁶ valine,¹⁷ isoleucine¹⁸ and leucine¹⁹) present in gas phase two dominant conformers stabilized by either a bifurcated $N-H\cdots O=C$ hydrogen bond with a *cis*-COOH configuration (type I) or a $N\cdots H-O$ hydrogen bond (type II). In contrast, the presence of polar functional groups in the α -amino acids side chains dramatically increases the number of conformers allowing observation of conformers with the third kind of hydrogen bond, $N-H\cdots O-H$ (type III). Thus, six conformers were detected for cysteine²⁰ and aspartic acid²¹ and seven for serine²² and threonine.²³ However, these general trends observe two exceptions: asparagine²⁴ and proline.²⁵ Asparagine²⁴ adopts one dominant structure in sharp contrast with the expected multi-conformational behavior for other polar side chains-containing amino acids. Such has been explained by an intramolecular hydrogen bonding network structure. Proline is a singular proteinogenic amino acid due to its side chain being closed into a pyrrolidine ring. It has been shown¹⁴ that proline adopts four different conformations in the gas phase, two type-I and two type-II conformers with both *C γ -endo* and a *C γ -exo* pyrrolidine ring bent configurations. Thus, the presence of a pyrrolidine ring in the proline's side chain not only does not restrict its flexibility but it increases the number of observed conformations.

Pipecolic acid, shown in Figure 1, is a non-proteinogenic α -amino acid whose side chain is closing a piperidine ring. Consequently, pipecolic acid shares with proline its structure differing solely in the ring size, 6-membered ring opposed to the 5-membered ring of proline. The similar structure usually means comparable properties, and this is true in this case. Pipecolic acid plays a vital role as a proline substitute in numerous peptidomimetic syntheses²⁶ or the design of potential anti-rheumatoid arthritis drugs like synthetic MHC class II ligands.²⁷ This can be understood as the nature of the fundamental intermolecular interactions in which proline and pipecolic acid are involved does not change noticeably. However, what happens with their respective intrinsic intramolecular forces? How does the increase in the ring size influence the overall conformational panorama?

In the pursuit to answer such questions, we have addressed the task of elucidating the conformational preferences of the isolated L-pipecolic acid molecule using the benefits of laser ablation combined with both narrow- and broad-band Fourier-transform microwave spectroscopy (LA-MB-FTMW and LA-CP-FTMW) techniques.^{11,28,29} As far as we could ascertain from the literature, no works on the conformational characterization of L-pipecolic

acid have been published until now. As such, the present work represents the first reliable information related to the structural properties of this biologically active molecule.

Methods

Experimental details

The rotational spectrum of L-pipecolic acid was first investigated using a LA-CP-FTMW spectrometer²⁸ operating in the 6–12 GHz frequency region. Commercial samples of L-pipecolic acid (m.p. 279°C) were used without any further purification to form solid rods by pressing the compound's fine powder mixed with a small amount of commercial binder. These rods were placed in the ablation nozzle and a picosecond Nd:YAG laser (20 mJ per pulse, 35 ps pulse width) was used as a vaporization tool. The resulting products of the laser ablation were supersonically expanded utilizing the flow of neon (10 bar) gas and then probed by CP-FTMW spectroscopy. Details of the experimental setup have been given elsewhere.²⁸ Chirped-pulses of 4 μ s directly generated by the 24 Gs/s AWG were amplified to about 300W peak power using a traveling wave tube amplifier. A parabolic reflector system composed of dual ridge horns and two parabolic reflectors in a paraxial beam configuration³⁰ was used to broadcast the excitation pulse and receive the broadband molecular emission. At a repetition rate of 2 Hz, a total of 90 000 free induction decays (4 FID emissions per gas pulse) each with 10 μ s length duration were averaged and digitized using a 50 GS/s digital oscilloscope. The frequency domain spectrum in the 6–12 GHz frequency range was obtained by taking a fast Fourier transform (FFT) following the application of a Kaiser–Bessel window to improve the baseline resolution.

The sub-Doppler resolution of the LA-MB-FTMW technique,²⁹ operating from 6 to 18 GHz, was used to record the rotational spectrum with the resolution necessary to analyze the hyperfine structure due to the presence of a ^{14}N nucleus in the molecule. A short microwave radiation pulse of 0.3 μ s duration was applied to polarize all the vaporized molecules. The registered free induction decay was then converted to the frequency domain by Fourier transformation. All the transitions appeared as Doppler doublets due to the parallel configuration of the molecular beam and the microwave radiation. The resonance frequency was determined as the arithmetic means of the two Doppler components. Frequency accuracy better than 3 kHz and an estimated resolution of 5 kHz are achieved in the experiment. From 50 to 100 averages were phase-coherently coadded to achieve reasonable signal to noise ratios (S/N).

Theoretical Modelling

The conformational landscape of L-pipecolic acid was explored with the help of quantum chemical calculations.³¹ As seen in Figure 1 pipecolic acid possesses a piperidine ring which can assume either of the two chair $^a\text{C}_6$ or $^b\text{C}_a$ configurations. The position of the hydrogen atom of the amine moiety contributes to adding another degree of freedom to the conformational panorama of pipecolic acid. Thus, for each of the ring configurations, the hydrogen atom may be in *equatorial* (*e*) or *axial* (*a*) position resulting four different configurations denoted as; $^a\text{C}_6\text{-e}$, $^a\text{C}_6\text{-a}$, $^b\text{C}_a\text{-e}$, and $^b\text{C}_a\text{-a}$. Using these four configurations as starting points, we examined the plausible conformations associated with all the different

orientations of the $-\text{COOH}$ moiety. In this manner, we found 16 conformational species that were optimized using the MP2 method and a 6-311++G(d,p) basis set.³² Each conformer was confirmed to be a local minimum in the potential energy surface by checking that its Hessian matrix did not have any imaginary eigenvalues. Besides the relative electronic and Gibbs free energies of the conformers, the molecular properties relevant to guide the spectral assignments, namely rotational constants, and dipole moment components, were extracted from the theoretical calculations. The molecular structures of the low-lying energy conformers (below 900 cm^{-1}), which will be sufficiently populated in the supersonic jet and will thus be the most promising candidates to be observed in the rotational spectrum, are shown in Table 1 together with their respective molecular parameters. The data for the rest of the predicted conformers are collected in Table S1 and their structures in Figure S1 of the Supplementary Material. The nomenclature used to designate each conformer was based on the two indexes above and an additional one to label the type of hydrogen bond between the amino and carboxylic groups, type-I ($\text{N}-\text{H}\cdots\text{O}=\text{C}$), type-II ($\text{N}\cdots\text{H}-\text{O}$) or type-III ($\text{N}-\text{H}\cdots\text{O}-\text{H}$).

Results and Discussion

The broadband recorded spectrum is very rich, and the data analysis was complicated due to overlapping contributions from the individual pipecolic acid conformers. Figure 2 depicts we show a portion of the measured rotational spectrum (upper trace, in black) plotted against the fitted rotational lines for all observed conformations of pipecolic acid (lower traces, in colors). The initial assignment of experimental lines to rotational transitions of a certain conformer was performed *via* a fitting procedure based on Watson's A-reduced rigid rotor Hamiltonian as implemented in JB95 program package.³³ A total of nine conformers of pipecolic acid were observed with comparable transition intensities. Although several lines remained unassigned in the spectrum, as can be seen in the Figure S2, identification of further rotamers could not be achieved.

Pipecolic acid possesses one ^{14}N nucleus ($I=1$) 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}N nuclear spin ($I=1$) and the overall angular momentum, giving rise to a hyperfine structure in the rotational spectrum. As can be seen in Figure 3, the resolution attained with the CP-FTMW technique is not sufficient to observe and analyze this hyperfine structure. This is consistent with our previous works where we have shown that the hyperfine splitting (few kHz) for heavy molecular systems like pipecolic acid cannot be resolved by the present CP-FTMW experiments.^{34–36} For this reason, in a second stage of the investigation pipecolic acid was probed using our LA-MB-FTMW technique, which provides the high resolution needed to fully resolve this hyperfine structure (as shown in Figure 3). All the observed rotational frequencies for the nine species (see Tables S2-S10 of the Supplementary Material) were analyzed³⁷ using a Watson's A-reduced Hamiltonian $H = H_R + H_Q$, where H_R is the semirigid rotor Hamiltonian and H_Q describes the nuclear quadrupole coupling interaction.^{38,39} The energy levels involved in each transition were thus labelled with the quantum numbers J , K_{-J} , K_{+J} and F . The analysis rendered the accurate experimental rotational and nuclear quadrupole coupling constants shown in Table 2.

The analysis of the inertial data provided the first piece of evidence in the assignment of the pipecolic acid rotamers. In the pipecolic acid, the *ab* inertial plane lies close to the piperidine ring atoms and the planar moment, $P_c = \frac{1}{2} (I_a + I_b - I_c) = m r_c^2$, gives the mass extension out of the *ab* inertial plane. According to the values of planar moments, the predicted low-energy conformers can be classified into three groups that differ in the arrangement of the –COOH moiety with respect to the *ab* inertial plane. In the aC_8 family of conformers, the C_α -COOH bond lies equatorially to the plane, and thus these species show the lowest P_c values. Furthermore, this family can be broken down into two groups. In the first one, aC_8 -e-I, aC_8 -a-II and aC_8 -e-III, the carboxylic moiety lies in the plane of the ring, which contributes to a relatively small value of P_c ; whereas in the other group, aC_8 -a-I, aC_8 -e-II and aC_8 -a-III, the carboxylic group is rotated in such a way that it is almost perpendicular to said plane, thus increasing P_c value when compared to the previous group. Comparison between the experimental values of P_c for all the rotamers with those calculated for the first group (15.4 for aC_8 -e-I, 14.7 for aC_8 -a-II and 16.9 for aC_8 -e-III) points to the assignment of these conformers as rotamers VI (15.8), II (14.8) and VII (16.2), respectively. The final assignment is achieved by comparing the experimental and the predicted rotational and nuclear quadrupole coupling constants for these species, and the excellent agreement between them confirms the initial identification. This is reinforced by the type of spectra observed for each rotamer (*b*- and *c*-type for both VI and VII and *a*- and *b*- type for II) that agree with the predicted values of the dipole moment components. Using the same strategy, rotamers III, IV and V were assigned to conformers aC_8 -e-II, aC_8 -a-I and aC_8 -a-III, respectively.

The remaining rotamers, I and VIII and IX present the highest P_c values, what indicates the larger mass out of the *ab* plane. Hence, these rotamers can be ascribed as conformers of ${}^\delta C_\alpha$ -family, in which the carboxylic moiety is in an axial position about the piperidine ring. Comparison of the experimental P_c values, 54.9, 59.0 and 58.2, with those predicted for conformers ${}^\delta C_\alpha$ -a-II (55.0), ${}^\delta C_\alpha$ -e-III (59.8) and ${}^\delta C_\alpha$ -e-I (59.2) allows us to identify rotamer I as ${}^\delta C_\alpha$ -a-II conformer. This assignment is confirmed by the good agreement found between the experimental and predicted rotational and nuclear quadrupole coupling constants as well as by the type of spectrum observed, *a*-, *b*- and *c*-type, which is consistent with the dipole moment component values calculated for the conformer above. The P_c value of rotamers VIII and IX are compatible with both for ${}^\delta C_\alpha$ -e-III and ${}^\delta C_\alpha$ -e-I conformers due to these species only differ in the orientation of the carboxylic group, which supposes a small change in the mass distribution of the species. While rotational constants are strongly related to mass distribution, nuclear quadrupole coupling interactions depend critically on the electronic environment, position and orientation of the ${}^{14}\text{N}$ nucleus. Thus, small changes in the molecular structure can be sensed through the variation of the nuclear quadrupole coupling constants which allow to unambiguously distinguishing conformers when the rotational constant cannot be used. So, making use of the quadrupole coupling constants rotamer VIII was identified as conformer ${}^\delta C_\alpha$ -e-III and rotamer IX as conformer ${}^\delta C_\alpha$ -e-I.

The identified conformers for pipecolic acid all exhibit intramolecular hydrogen bonds between the carboxylic and amine moieties. These interactions include the three types of intramolecular hydrogen bonds observed in previous studies of amino acids, type-I, -II and -III. However, the observation of type-III conformers for pipecolic acid is an unexpected

fact because no type-III conformers were observed for the analogous amino acid proline. As we mentioned above, type-I and -II have been found for polar and nonpolar amino acids while type-III have only been observed in polar amino acids. Conformers with type-I and type-III differ mainly in the orientation of the COOH group, which is rotated by *ca.* 180° around the C_α-C(O) bond. Interconversion between them may, therefore, occur, provided that the energy barrier is low enough. This process takes place in the early stages of the supersonic expansion by collisions between the different conformers and the buffer gas molecules and it has been deduced⁴⁰ that collisional removal of a higher energy conformer is possible when the isomerization barrier is lower than 400 cm⁻¹. In nonpolar amino acids, this barrier height is lower than 400 cm⁻¹ and the interconversion occurs. In contrast, for polar amino acids this barrier is higher, due to interactions between the -COOH moiety and the polar side chain, the interconversion does not occur and the type-III conformers can be observed.^{20–23} The explanation for the different conformational behavior for pipecolic acid with respect to proline can be found in the previous study of Ac₃c (1-aminocyclopropane carboxylic acid) where it was hypothesized⁴¹ that α-amino acids lacking a polar side chain but exhibiting structural features leading to hindered rotation about the C_α-C(O) bond would also present a type-III form amenable to detection. This was corroborated by the experimental evidence that indeed such form was present for this molecule. Interestingly, it was found that the presence of a cyclopropane moiety was effective in hindering the III→I relaxation process and the energy barrier associated with the III→I interconversion was estimated to be higher than 1800 cm⁻¹. Based on this, we calculated the potential energy profile between the pairs of conformers ^aC_δ-e-III/^aC_δ-e-I, ^aC_δ-a-III/^aC_δ-e-I and ^δC_α-e-III/^δC_α-e-I. (Figures 4 and S3). Barrier heights of 680, 880 and 707 cm⁻¹ respectively were found, which are high enough to hamper conformational relaxation justifying the observation of type-III conformers for pipecolic acid. These values for the barrier height are higher than those calculated for proline conformers (~300 cm⁻¹). The change in the barrier height can be attributed to an increase in the steric hindrance produced by the larger size of the piperidine ring.

The results presented in this work show that the conformational landscape of pipecolic acid in the gas phase is composed at least by nine conformations. At this point, the initial questions about whether the increase of one carbon atom in the side chain affects to the intramolecular interactions and conformational behavior of pipecolic acid compared to those of proline can be answered. First, the intramolecular interactions found for pipecolic acid are of the same nature than those for proline, between the amino and the carboxylic groups. However, as it was mentioned above, type-III intramolecular hydrogen bonds have been observed for pipecolic acid and not for proline due to the influence of the piperidine ring. Second, the number of pipecolic acid conformations is more than two times the observed for proline. Again, this is an effect produced by the increase of the ring size (flexibility) that lead to four low energy ring configurations, which in turn multiply the number of accessible conformers

Conclusions

The molecular shape of pipecolic acid has been investigated in the gas phase for the first time, leading to an overview of its conformational panorama. Nine different structures of

this amino acid have been observed in the rotational spectrum. The provided data, obtained through rotational spectroscopy, as well as the possibility of directly correlate them with data obtained by high-level computational methods, makes this technique an unmatched tool for unambiguous conformational ascription.

Notable differences have been found between the behavior of pipecolic acid and that shown by the other nonpolar amino acids studied so far, also with its analog proline: the number of observed conformations is increased and conformers with type-III hydrogen bonds between the amino and carboxylic groups have been observed. The former has to do with the flexibility imparted by the piperidine ring in the side chain and the second one with the fact that the steric hindrance associated with the rotation of the carboxylic moiety in pipecolic acid is higher than in other nonpolar amino acids. Such difference, albeit slight, produce increments in the interconversion barriers that are enough to prevent relaxation of the type-III forms into the energetically more favorable type I forms.

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Figure 1.
Plausible configurations adopted by L-pipecolic acid molecule depending on the chair conformation and axial or equatorial arrangement of the amino group

Figure 2.

A section of the rotational spectrum of pipecolic acid. The upper trace (in black) shows the experimental spectrum. The lower traces (in colour) represent spectral simulations employing the fitted parameters of pipecolic acid given in Table 2. Intensities are based on calculated dipole moment components and estimated relative populations.

Figure 3.

Nuclear quadrupole hyperfine structure of $4_{14} \leftarrow 3_{03}$ and $2_{20} \leftarrow 1_{11}$ rotational transitions of rotamers VI and XI; (upper) experimental by LA-CP-FTMW spectroscopy; (middle) experimental by LA-MB-FTMW spectroscopy. (Bottom) The simulated hyperfine structure using the experimentally calculated nuclear quadrupole coupling constants in Table 2 for VI and XI conformers, respectively.

Figure 4. Potential energy profile of ${}^a\text{C}_\delta\text{-a-III}$ and ${}^a\text{C}_\delta\text{-a-I}$ conformers of pipecolic acid around the $\angle\text{NCCO}$ dihedral angle calculated at MP2/6-311++G (d, p) level of theory.

Table 1
Theoretical spectroscopic constants for the nine lowest energy conformers of pipecolic acid calculated at MP2/6-311++G (d, p) level of theory.

	${}^a\text{C}_{\delta\text{-a-I}}$	${}^a\text{C}_{\alpha\text{-e-I}}$	${}^a\text{C}_{\delta\text{-e-I}}$	${}^a\text{C}_{\alpha\text{-a-II}}$	${}^a\text{C}_{\delta\text{-e-II}}$	${}^a\text{C}_{\delta\text{-a-II}}$	${}^a\text{C}_{\delta\text{-e-III}}$	${}^a\text{C}_{\delta\text{-a-III}}$	${}^a\text{C}_{\alpha\text{-e-III}}$
A^a	3247	2562	3264	2544	3221	3199	3252	3248	2536
B	1094	1425	1142	1447	1123	1159	1144	1084	1445
C	942	1166	892	1154	959	895	897	961	1177
P_c	40.6	59.2	15.4	55.0	40.0	14.7	16.9	48.0	59.8
$ \mu_a $	1.1	0.3	0.7	4.4	5.1	5.1	0.8	1.9	0.3
$ \mu_b $	1.8	1.1	1.2	1.5	1.2	1.2	1.4	0.2	1.1
$ \mu_c $	0.1	0.3	1.2	2.2	0.6	0.1	1.2	1.8	0.3
N/χ_{aa}	1.60	-0.59	2.63	-1.31	2.29	0.49	2.51	1.35	-1.21
N/χ_{bb}	-0.24	2.26	2.48	-0.78	1.98	-1.71	2.21	-0.45	2.59
N/χ_{cc}	-1.35	-1.67	-5.10	2.09	-4.27	1.22	-4.72	-0.90	-1.38
E^b	138	40	95	0	181	336	210	446	161
G^c	20	1	0	49	182	351	102	330	118

^a A , B , and C represent the rotational constants (in MHz); μ_a , μ_b , and μ_c are the electric dipole moment components (in D); P_c is the planar inertia moment, in $\text{u}\text{\AA}^2$ (conversion factor: $505379.1 \text{ MHz}\cdot\text{u}\text{\AA}^2$); χ_{aa} , χ_{bb} , and χ_{cc} , are the diagonal elements of the ^{14}N nuclear quadrupole coupling tensor (in MHz);

^b Relative energies (in cm^{-1}) with respect to the global minimum.

^c Relative Gibbs free energies (in cm^{-1}) calculated at 298 K.

Table 2
Experimental spectroscopic parameters for the nine observed conformers of pipecolic acid.

	Rotamer I	Rotamer II	Rotamer III	Rotamer IV	Rotamer V	Rotamer VI	Rotamer VII	Rotamer VIII	Rotamer IX
A^a	2546.08754(57) ^d	3199.4725(16)	3217.1596(18)	3244.1988(13)	3242.2075(16)	3264.61235(69)	3253.5003(14)	2545.15566(52)	2578.23929(50)
B	1437.99330(58)	1155.5398(10)	1121.3978(11)	1101.02769(88)	1089.45650(46)	1137.97954(49)	1141.39237(90)	1431.66090(64)	1407.89007(67)
C	1148.37819(72)	893.34360(65)	953.9595(11)	933.69369(51)	951.76440(69)	890.77335(60)	893.37688(67)	1165.58811(49)	1152.28778(52)
J	0.185(12)	0.0260(34)	0.0471(64)	0.0613(60)	0.0576(87)	0.0232(51)	0.0349(50)	0.1815(70)	0.2042(70)
P_c	54.9	14.8	38.9	36.7	44.4	15.8	16.2	59.0	58.2
$ \mu_a $	observed	observed	observed	observed	observed	observed	observed	not observed	not observed
$ \mu_b $	observed	observed	observed	observed	not observed	observed	observed	observed	observed
$ \mu_c $	observed	not observed	not observed	not observed	observed	observed	observed	not observed	not observed
N/χ_{aa}	-1.2363(25)	0.555(15)	2.2491(34)	1.6861(53)	1.426(22)	2.5865(27)	2.4635(70)	-1.1167(31)	-0.5280(33)
N/χ_{bb}	-0.8201(34)	-1.668(10)	1.9803(91)	-0.1724(66)	-0.319(24)	2.3922(35)	2.1768(69)	2.5324(41)	2.1982(41)
N/χ_{cc}	2.0564(34)	1.112(10)	-4.2293(91)	-1.5136(66)	-1.107(24)	-4.9787(35)	-4.6404(69)	-1.4157(41)	-1.6702(41)
N^b	35	19	20	21	23	28	20	30	29
σ^c	2.4	1.1	2.1	2.2	2.8	1.9	1.7	3.0	2.7

^a A , B , and C represent the rotational constants (in MHz); μ_a , μ_b , and μ_c are the electric dipole moment components (in D). J represents the centrifugal distortion constants (in kHz); P_c is the planar inertia moment, in $\text{u}\text{\AA}^2$ (conversion factor: $505379.1 \text{ MHz}\cdot\text{u}\text{\AA}^2$); χ_{aa} , χ_{bb} , and χ_{cc} are the diagonal elements of the ^{14}N nuclear quadrupole coupling tensor (in MHz);

^b Number of measured transitions.

^c RMS deviation of the fit (in kHz).

^d Standard error in parentheses in units of the last digit.