

The Gas-Phase Structure of Alanine

Susana Blanco, Alberto Lesarri, Juan C. López, and José L. Alonso*

Contribution from the Grupo de Espectroscopía Molecular (GEM), Departamento de Química Física y Química Inorgánica, Facultad de Ciencias, Universidad de Valladolid, 47005 Valladolid, Spain

Received March 24, 2004; E-mail: jalonso@qf.uva.es

Abstract: The jet-cooled rotational spectrum of neutral alanine has been studied using laser-ablation molecular-beam Fourier transform microwave spectroscopy (LA-MB-FTMW). The spectra of the two most stable forms were observed in the frequency range 6–18 GHz for the parent, ^{15}N alanine, three single ^{13}C species, and four single D species. The ^{14}N nuclear quadrupole coupling hyperfine structures have been resolved, and their comparison with those calculated theoretically confirms unambiguously the conformer assignments. The independent structures of both conformers have been determined experimentally for the first time using r_s and r_0 procedures. In both cases, the amino acid backbone is nonplanar. For the most stable conformer I, the COOH group adopts a cis configuration and an asymmetric bifurcated hydrogen bond is formed between the amino group and carbonyl oxygen ($r(\text{N}-\text{H}_a \cdots \text{O}=\text{C}) = 2.70(2)$ Å and $r(\text{N}-\text{H}_b \cdots \text{O}=\text{C}) = 2.88(2)$ Å). For conformer IIa, the COOH group adopts a trans configuration and is stabilized by a $\text{O}-\text{H} \cdots \text{N}$ hydrogen bond ($r(\text{O}-\text{H} \cdots \text{N}) = 1.96(2)$ Å). The relative conformer abundances in the supersonic expansion have also been investigated.

I. Introduction

The study of amino acids ($\text{H}_2\text{N}-\text{CH}(\text{R})-\text{COOH}$) under isolation conditions in gas phase is of great chemical relevance in connection with their biochemical role¹ and the origin of life.² The function of amino acids depends strongly on their structure and its changes during biological processes. Since solid or diluted amino acids are involved in a great number of intermolecular interactions that result in ionized zwitterionic structures ($^+\text{H}_3\text{N}-\text{CH}(\text{R})-\text{COO}^-$), the isolated gas-phase neutral amino acids represent the best approximation to understand the inherent properties of these protein building blocks.

Amino acids are very flexible molecules, and their conformational landscape is characterized by a delicate balance of covalent and noncovalent interactions (especially hydrogen bonding), which may result in a large number of low-energy conformers. The use of supersonic jets³ constituted a great progress for the isolation and detection of these conformers. The study of the gas-phase rotational spectra of these jet-cooled amino acids provides detailed structural information for each of the individual conformations with significant populations in the adiabatic expansion and allows the characterization of the intramolecular interactions that stabilize each conformer.^{4,5} In addition, the investigation of the rotational spectra of amino acids is of enormous interest for their radioastronomical detection. The recent discovery of interstellar glycine⁶ from its

rotational spectrum has strengthened the thesis that interstellar organic molecules could have played a pivotal role in the prebiotic chemistry of early Earth.²

The experimental studies of gas-phase amino acids have been hindered by their high melting points and associated low vapor pressures which, combined with a low thermal stability, makes many of these compounds decompose before melting. In fact, the use of thermal heating only allowed the rotational study of the natural amino acids of glycine⁷ (mp 240 °C) and alanine⁸ (mp 315 °C). Fortunately, laser desorption⁹ has proven to be a suitable way to overcome this problem and since the first experiments¹⁰ is being applied successfully in combination with laser spectroscopy techniques to the study of neutral molecules

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of biological interest.¹¹ We have developed a spectrometer that combines laser ablation of organic solid samples with microwave spectroscopy in a supersonic jet.¹² This technique, known as laser-ablation molecular-beam fourier transform microwave spectroscopy, LA-MB-FTMW, has been applied successfully to other amino acids, such as proline⁴ (mp 228 °C) and valine⁵ (mp 295–300 °C), which were observed in the gas phase for the first time in our laboratory, and to several organic solids such as 1,3,5-trithiane¹³ and thiourea.¹⁴ For proline,⁴ two conformers with a N···H–O intramolecular hydrogen bond were found, while for valine,⁵ two conformers with N–H···O=C and N···H–O intramolecular hydrogen bonds were found.

Glycine and alanine are the simplest natural α -amino acids, and they were the first neutral amino acids to be studied in gas phase by their rotational spectrum. The conformational landscape and structure of glycine is now well understood thanks to a sustained experimental⁷ and theoretical work.¹⁵ However, the work done on alanine is much more limited, and a revision is advisable. The first experimental studies of alanine in solid phase¹⁶ revealed a zwitterionic form (as evidenced by the small difference between the two carbon–oxygen distances and the positions of the H atoms in the ammonium group). A free-jet rotational study conducted in the millimeter-wave region by Godfrey et al.⁸ established the main conformational features of the neutral molecule in gas phase and verified the existence of two alanine conformers and their relative abundances in the supersonic jet. Up to 13 possible conformers were predicted from ab initio computations.^{8,17,18} Those with the lowest energy are shown in Figure 1. The two conformers observed experimentally by Godfrey⁸ are denoted as conformers I and IIa. Conformer I exhibits an intramolecular hydrogen bond between the oxygen atom of the carbonyl group and the hydrogen atoms of the amino group (NH···O=C). In conformer IIa, an intramolecular hydrogen bond is established between the hydrogen atom of the hydroxyl group and the N atom of the amino group (N···HO). A large amplitude motion associated to small torsions

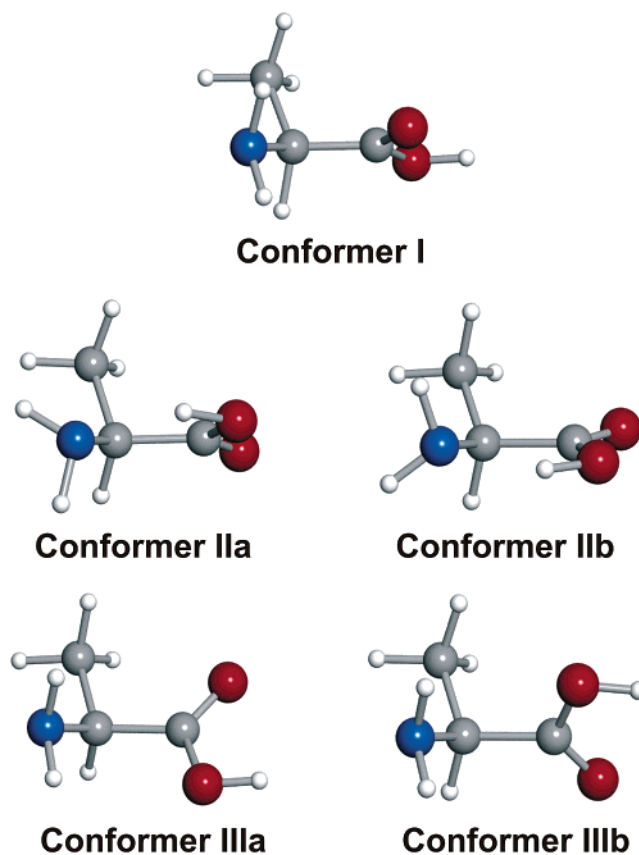


Figure 1. Lowest-energy conformers of alanine.

of the carboxylic and amino groups can give rise to a second conformer with an intramolecular hydrogen bond of the type N···HO, which is denoted as conformer IIb. A third type of intramolecular hydrogen bond, denoted as III, can be formed between the amino group and the oxygen atom of the carboxyl group (NH···O–H) and gives rise to conformers IIIa and IIIb. This third type of hydrogen bond has not yet been observed in the gas phase.

There are several motivations to investigate the rotational spectra of alanine. First, the rotational work of Godfrey⁸ was completed in the millimeter-wave region and could not resolve the ¹⁴N quadrupole coupling hyperfine structure of the molecule. With the development of our LA-MB-FTMW spectrometer, the problem of sample vaporization is solved and the rotational spectrum is recorded with unparalleled sub-Doppler resolution. The nuclear quadrupole coupling parameters are very sensitive to both the electronic environment of the nitrogen atom and its orientation with respect to the principal inertial axis,¹⁹ and thus they could serve to unambiguously determine the identity of the observed conformers.⁵

Second, the experimental information on the structure of gas-phase alanine is relatively poor. The only structural work done thus far on gas-phase alanine was based on the analysis of electron diffraction data.²⁰ This analysis, even if combined with the rotational constants reported by Godfrey et al.,⁸ was incomplete since it was based on the assumption that the vapor of alanine is composed only of conformer I. As is well-known,

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the different gas-phase low-energy conformers of a molecular system give rise to independent rotational spectra. As a consequence, the structure of each conformer can be independently obtained from the spectra of different isotopomers. In the present work, the spectra of the parent, ^{15}N , ^{13}C , and several deuterated species of alanine have been investigated to elucidate the structure of the possible intramolecular hydrogen bonds and other relevant conformational details.

Finally, this work gives us the chance to reassess the possibility of observing other conformers of alanine. The lack of observation of conformers IIb and IIIa/IIIb in the supersonic jet was attributed to selective collisional relaxation processes in the jet associated to low interconversion barriers.¹⁷ It is accepted that this event takes place by collisions with the noble carrier gas in the adiabatic expansion if the barrier to interconversion between the different conformers is low enough. This phenomenon, which is enhanced for heavy carrier gases as Ar, has been observed in systems involving only one degree of freedom such as torsional isomerism²¹ and axial-equatorial relaxation in hydrogen bond complexes²² when barriers are less than about 400 cm^{-1} . Studies on systems with multiple degrees of freedom have proposed barrier heights below 1000 cm^{-1} .²³ The previous jet-cooled millimeter-wave rotational study of alanine was performed using Ar as carrier gas. The use of lighter carrier gases such as Ne or He could allow us to observe new conformers.

II. Methods

A. Experimental. The details of the LA-MB-FTMW technique have been given elsewhere,¹² and therefore only a brief description of the spectrometer is given here. In this experiment, the alanine molecules were vaporized by laser ablation. The ablation products, diluted in a light inert carrier gas, expanded supersonically to form a molecular beam between the mirrors of a Fabry–Pérot resonator where they were probed using Fourier transform microwave spectroscopy. The high-vacuum chamber was recently enlarged to accommodate new resonator mirrors with a diameter of 55 cm. Laser ablation was produced by the second harmonic (512 nm) of a Q-switched pulsed Nd:YAG laser. The green laser pulses were focused onto the desorption nozzle, where a solid sample rod was held vertically at the exit channel of a commercial solenoid-pulsed valve. The whole desorption nozzle was located on the backside of one of the Fabry–Pérot mirrors, close to the resonator axis. It could be extracted from the high-vacuum chamber without stopping the pumping system thanks to an auxiliary evacuated chamber that could be isolated by a gate valve. The collinear arrangement of the supersonic jet and the resonator axis resulted in a larger section of the molecular beam being probed by the microwave field, and accordingly, the sensitivity of the spectrometer increased. The samples were pressed from alanine powder (mixed with minimum quantities of a commercial binder) to form a solid rod of about 6-mm width. The target rod rotated and translated so the laser hit a different point of the sample surface in each successive pulse. We used commercial samples for the parent species (Aldrich, DL-alanine, 99%) and the observed isotopomers DL-alanine- ^{15}N (Isotec, 99%), DL-alanine-1- ^{13}C (Isotec, 99%), DL-alanine-2- ^{13}C (Cambridge Isotope Labs, 99%), DL-alanine-

3- ^{13}C (Isotec, 99%), DL-alanine-3,3,3- D_3 (Isotec, 99%), and DL-alanine-2- D_1 (Isotec, 98%). The monodeuterated species at the amino (NDH- and NHD-alanine) and carboxylic (COOD-alanine) groups were prepared in situ dissolving DL-alanine in D_2O followed by recrystallization. Each measuring cycle starts with a gas pulse (tip. 0.5 ms) of the carrier gas, which is followed by a laser hit on the solid sample. Argon, neon, or helium at stagnation pressures of 3–5 bar were alternatively used as carrier gases. After the molecular beam has been created, a very short (tip. $0.4\ \mu\text{s}$) microwave pulse polarized the species in the jet. When the excitation microwave pulse finished, a very low-noise microwave detection system was used to record the molecular emission signal in the time domain. The emission signal was down-converted to the RF region around 7 MHz by a two-step superheterodyne converter, where it was digitized with a 25 MHz A/D transient recorder. Finally, the frequency domain spectrum containing the rotational transitions was obtained from the Fourier transformation of the time domain signal. Because of the collinearity between the supersonic jet and the microwave resonator axis, each line in the spectrum appeared as a Doppler doublet, and the transition frequencies were taken as the arithmetic mean of the Doppler components. Typical line widths at half-maximum were better than 7 kHz; frequency accuracy was below 3 kHz.

B. Computational. Nuclear quadrupole coupling constants were useful to identify unambiguously the observed conformers of gas-phase amino acids. This was shown recently for valine⁵ by comparison of the observed ^{14}N quadrupole coupling constants with those calculated from theoretical methods. Ab initio computations were conducted on the lowest-energy conformers^{8,17,18} of alanine (Figure 1) using second-order Møller–Plesset perturbation theory (MP2), within the frozen core approximation, and a standard 6-311++G(d,p) basis set.²⁴ The quadrupole coupling constants were difficult to obtain accurately from ab initio computations,²⁵ but for the scope of discriminating the different conformers of alanine the level of theory used here was sufficient. This level of theory proved also to yield reasonable predictions of other spectroscopic properties.⁵ The predicted values for each conformer are collected in Table 1, together with their energies and other relevant molecular properties such as rotational constants and electric dipole moment. The electronic energies given in Table 1 were calculated at the MP4(SDTQ) level for the MP2 optimized structures. Further calculations of the harmonic zero-point energies (ZPE) and free energies ($G_{298\text{K}}$ and $G_{498\text{K}}$) were done at the MP2/6-311++G(d,p) level by means of analytic Hessian matrix calculations. Conformer IIIb does not appear in Table 1 since it did not correspond clearly to a minimum at the

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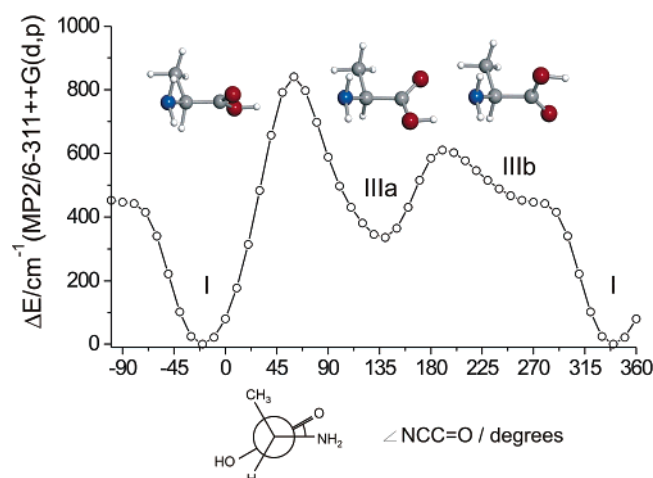
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Table 1. Predicted Spectroscopic Parameters for the Lowest-Energy Conformers of Alanine (see Figure 1) from ab Initio MP2/6-311++G(d,p) Calculations

	I	IIa	IIb	IIIa
$E_{MP4}/\text{hartree}$	-323.0694782	-323.0689726	-323.0686408	-323.0679504
$\Delta E_{MP4}^a/\text{kJ mol}^{-1}$	0	1.33	2.20	4.01
$\Delta E_{MP4}^a + \text{ZPE}/\text{kJ mol}^{-1}$	0	2.91	3.57	4.33
$\Delta G_{298\text{K}}^b/\text{kJ mol}^{-1}$	0	4.73	4.52	4.49
$\Delta G_{498\text{K}}^b/\text{kJ mol}^{-1}$	0	6.73	5.88	4.70
A/MHz	5074	4993	4862	5068
B/MHz	3051	3197	3441	2841
C/MHz	2298	2344	2169	2492
χ_{aa}^c/MHz	-3.44	0.77	-1.39	-3.79
χ_{bb}^c/MHz	1.99	0.30	-0.51	2.23
χ_{cc}^c/MHz	1.46	-1.09	1.90	1.56
μ_a^d/D	0.64	5.91	5.24	0.30
μ_b^d/D	1.19	1.63	1.44	1.27
μ_c^d/D	0.42	0.83	0.40	1.19
μ_t^d/D	1.41	6.18	5.45	1.77

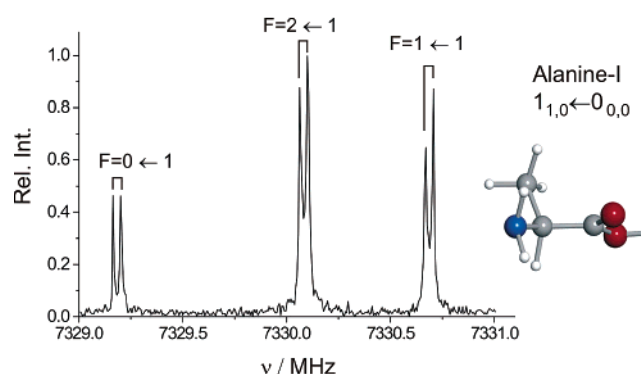
^a Electronic energies relative to conformer I have been calculated at the MP4(SDTQ)/6-311++G(d,p)//MP2/6-311++G(d,p) level. ^b Free energy relative to that of the most stable form calculated, at 298.15 and 498.15 K, by combining the MP4 energy results with those of MP2 harmonic model analytical frequency computations. ^c The ¹⁴N quadrupole coupling constants were obtained using the conversion: $\chi_{aa}^c/\text{MHz} = 2.34965 (Q/\text{fm}^2)(q_{aa}/\text{au})$, where q_{aa} is the corresponding element of the electric field gradient at N nucleus and the quadrupole coupling moment for ¹⁴N is taken to be $Q = 2.09 \text{ fm}^2$, after Cummings, P. L.; Bacskay, G. P.; Hush, N. S.; Aldrich, R. *J. Chem. Phys.* **1987**, *86*, 6908. Only diagonal elements are given. ^d Electric dipole moment. $1 \text{ D} \approx 3.3356 \times 10^{-30} \text{ C m}$.

**Figure 2.** Calculated MP2/6-311++G(d,p) energy profile of alanine along the torsional coordinate defined by dihedral angle $\angle\text{NCC}=\text{O}$.

frozen core MP2/6-311++G(d,p) level. This can be seen in Figure 2, where the calculated energy profile for the interconversion between conformers I and IIIa is represented.

III. Results and Discussion

A. Rotational Spectra. The rotational spectrum of conformer I (Figure 1) is that of an asymmetric rotor with dominant μ_b electric dipole moment transitions. The transitions corresponding to the parent species in the frequency region 6–18 GHz were readily located using the rotational parameters of Godfrey.⁸ A set of R-branch μ_a - and μ_b -type transitions were measured for this conformer. No μ_c -type transitions were observed. All transitions exhibited a well-resolved hyperfine structure (see Figure 3) characteristic of a nuclear quadrupole coupling interaction arising from a single ¹⁴N nucleus ($I = 1$). The relatively large microwave powers needed for the optimum polarization of the measured transitions agree with relatively weak μ_a and μ_b components. The measured rotational transitions were fitted²⁶ to the Watson's semirigid rotor Hamiltonian²⁷ H_R

**Figure 3.** $1_{1,0} \leftarrow 0_{0,0}$ rotational transition of conformer I for the parent ¹⁴N (upper trace) species of alanine. The ¹⁴N nuclear quadrupole coupling hyperfine components ($F' \leftarrow F''$) are labeled by the quantum numbers $F = I + J$. Each component appears as a doublet (\uparrow) because of Doppler effect (see Experimental Section).

using the asymmetric (A) or symmetric (S) reductions, supplemented with an additional term H_Q to account for the nuclear quadrupole coupling²⁸ caused by the ¹⁴N atom. We finally chose the A reduction (I' representation) on the basis of the quality of the different fits, the number of determinable parameters, and the observed correlations. In the final fit, all quartic centrifugal distortion constants could be determined for this conformer. We did not include the transitions measured by Godfrey⁸ et al. in our analysis, since line widths in their instrument are at least 40 times larger than that in our LA-MB-FTMW experiment and the nuclear quadrupole coupling hyperfine structure was not resolved. Splittings attributable to the internal rotation of the methyl group of alanine were not observed with the higher resolution of our instrument, setting a lower limit of 950 cm^{-1} for the methyl group torsional barrier. A fit of the observed transitions with a standard deviation below the estimated frequency accuracy of 3 kHz could be obtained with the only consideration of the diagonal elements of the ¹⁴N nuclear

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Table 2. Rotational Parameters for the Parent, ^{13}C , and ^{15}N Isotopomers of Conformer I of Alanine

	parent	$^{13}\text{C}_1$	$^{13}\text{C}_2$	$^{13}\text{C}_3$	^{15}N
A/MHz^a	5066.14560(42) ^d	5065.20500(48)	5057.32450(95)	4970.89132(56)	5017.29480(27)
B/MHz	3100.95058(29)	3091.28270(33)	3088.53147(73)	3067.18351(38)	3050.49311(25)
C/MHz	2264.01342(24)	2258.83518(27)	2258.67058(50)	2232.38563(31)	2229.20723(23)
Δ_J/kHz	2.452(13)	2.417(14)	2.423(15)	2.383(17)	2.331(13)
Δ_{JK}/kHz	−6.391(31)	−6.339(30)	[−6.391] ^e	−6.067(43)	−6.279(18)
Δ_K/kHz	5.410(79)	5.432(92)	[5.410]	5.20(10)	5.534(23)
δ_J/kHz	0.5696(31)	0.5709(34)	0.548(18)	0.5587(48)	0.5402(20)
δ_K/kHz	10.3777(54)	10.386(60)	10.66(11)	9.920(69)	10.013(20)
χ_{aa}/MHz	−3.2567(11)	−3.2636(14)	−3.2680(14)	−3.3493(14)	
χ_{bb}/MHz	2.0093(16)	2.0156(21)	2.0157(24)	2.1223(50)	
χ_{cc}/MHz	1.2474(16)	1.2480(21)	1.2523(24)	1.2270(50)	
σ/kHz^b	1.9	2.0	1.5	2.2	1.0
N^c	73	58	29	55	19

^a A , B , and C represent the rotational constants; Δ_J , Δ_{JK} , Δ_K , δ_J , and δ_K are the quartic centrifugal distortion constants; and χ_{aa} , χ_{bb} , and χ_{cc} are elements of the ^{14}N nuclear quadrupole coupling tensor. ^b rms deviation of the fit. ^c Number of transitions. ^d Standard error in parentheses in units of the last digit. ^e Parameters in square brackets were kept fixed in the fit.

Table 3. Rotational Parameters for the Deuterated Isotopomers of Conformer I of Alanine

	$\text{C}_2\text{-D}$	$\text{C}_3\text{-D}_3$	COO-D	N-D_3	N-D_2
A/MHz^a	4952.8925(12) ^d	4604.1195(67)	5052.178(12)	4961.296(59)	4889.2986(51)
B/MHz	3049.1367(16)	2925.5532(45)	2990.960(27)	3043.8558(29)	3051.1451(11)
C/MHz	2258.5517(14)	2122.4999(23)	2202.2684(18)	2222.3985(28)	2218.81838(75)
Δ_J/kHz	2.426(67)	[2.452]	[2.452]	[2.452]	[2.452]
Δ_{JK}/kHz	[−6.391] ^e	[−6.391]	[−6.391]	[−6.391]	[−6.391]
Δ_K/kHz	[5.410]	[5.410]	[5.410]	[5.410]	[5.410]
δ_J/kHz	[0.5696]	[0.5696]	[0.5696]	[0.5696]	[0.5696]
δ_K/kHz	10.92(43)	[10.3777]	[10.3777]	[10.3777]	[10.3777]
χ_{aa}/MHz	−3.3193(71)	[−3.2567]	[−3.2567]	[−3.2567]	[−3.2567]
χ_{bb}/MHz	2.011(12)	[2.0093]	[2.0093]	[2.0093]	[2.0093]
χ_{cc}/MHz	1.308(12)	[1.2474]	[1.2474]	[1.2474]	[1.2474]
σ/kHz^b	8.7	31.0	12.0	13.0	4.9
N^c	42	24	9	8	9

^a Parameter definition as in Table 2. ^b rms deviation of the fit. ^c Number of transitions. ^d Standard error in parentheses in units of the last digit. ^e Parameters in square brackets were kept fixed in the fit.

quadrupole coupling tensor $\chi_{\alpha\beta}$ ($\alpha, \beta = a, b, c$). The high accuracy of the experimental measurements indicates that the contribution to the observed frequencies from the nuclear quadrupole coupling tensor off-diagonal elements is negligible, and thus these parameters are not determinable. The final parameters for the parent species of conformer I are shown in Table 2. The rotational and centrifugal distortion constants are consistent with those previously reported.⁸ The determined quadrupole coupling constants are in good agreement with those calculated ab initio given in Table 1.

The analysis of conformer I was later extended to other isotopomers. The spectra of the monosubstituted 1- ^{13}C , 2- ^{13}C , 3- ^{13}C , ^{15}N , 2-CD, and 3-CD₃ species were assigned from the predictions of the expected isotopic substitution frequency shifts. For the ^{15}N ($I = 0$) species, no nuclear quadrupole coupling hyperfine effects are present. The monodeuterated species at the carboxylic (COOD) and amino groups (NHD, NDH) were also assigned with the same procedure. The analysis of the rotational transitions of the minor isotopomers was similar to the parent species, but not all centrifugal distortion constants could be determined in some cases. For all deuterated species the small nuclear quadrupole coupling effects due to the D atom were not considered since they were not completely resolved; the N nuclear quadrupole coupling parameters were fixed to those of the main species. The determined rotational parameters for the minor isotopomers are collected in Tables 2 and 3. The

observed rotational transitions of the 10 isotopomers assigned for conformer I of alanine are given as Supporting Information.

Conformer IIa (Figure 1) is an asymmetric rotor with a larger electric dipole moment, which is mainly oriented along the a principal inertial axis. The rotational spectrum for the parent species of this conformer was predicted with the rotational constants reported by Godfrey et al.,⁸ and the nuclear quadrupole coupling hyperfine structure arising from the ^{14}N atom was readily assigned (Figure 4). Finally, a set of R-branch μ_a -type and R- and Q-branch μ_b -type transitions were observed for conformer IIa. The observation of the μ_a -type spectrum of conformer IIa required much lower microwave powers and shorter excitation pulses than those needed to observe the corresponding lines of conformer I, confirming a large μ_a component for conformer IIa as observed experimentally.⁸ On the contrary, the μ_b -type transitions require the same relatively large microwave powers as needed to observe the μ_b -type spectra of conformer I. This is also in good agreement with the fact that both conformers have nearly equal μ_b electric dipole components.⁸ The study of conformer IIa was also extended to nine isotopomers, including all monosubstituted ^{13}C species, the ^{15}N species, and the monodeuterated species 2-CD, 3-CD₃, COOD, NHD, and NDH. The rotational transitions were analyzed as described above for conformer I, and the corresponding rotational parameters are collected in Tables 4 and 5. The full set of quartic centrifugal distortion constants could be

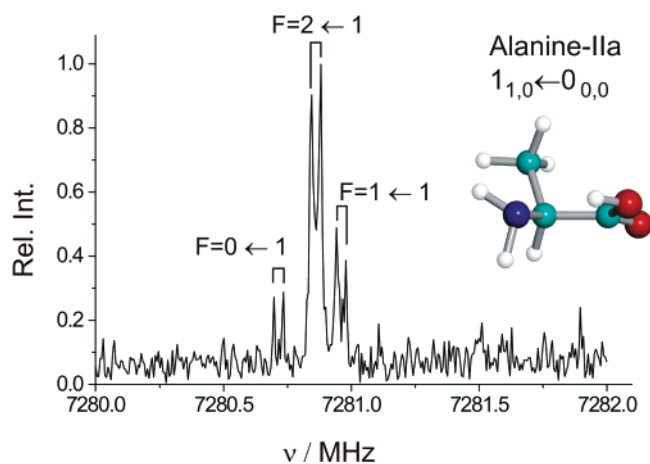


Figure 4. $1_{1,0} \leftarrow 0_{0,0}$ rotational transition of conformer IIa for the parent ^{14}N (upper trace) species of alanine. The ^{14}N nuclear quadrupole coupling hyperfine components ($F'' \leftarrow F'''$) are labeled by the quantum numbers $F = I + J$. Each component appears as a doublet (\square) because of Doppler effect (see Experimental Section).

determined only for the isotopomer with ^{15}N , and therefore, for the other species the non-determinable centrifugal distortion constants were fixed to the values obtained for this isotopomer. The whole set of new transitions measured for conformer IIa are collected as Supporting Information.

The quadrupole coupling constants χ_{aa} , χ_{bb} , and χ_{cc} determined for this conformer (see Tables 4 and 5) are only consistent with

those calculated ab initio for conformer IIa. As can be seen in Table 1, both the rotational constants and the electric dipole moment of conformers IIa and IIb are very similar, but the quadrupole coupling constants are markedly different because of the change in the orientation of the amino group with respect to the principal axis system of the molecule in going from conformer IIa to IIb.

Conformers IIIa and IIb were not found in the previous microwave work done by Godfrey et al.,⁸ and thus we decided to re-examine the possibility of finding these conformers using the higher sensitivity of our Fourier transform spectrometer. Conformer IIIa, with a computed energy of 4.33 kJ/mol relative to that of conformer I, is predicted to be an asymmetric rotor with a very small electric dipole moment component along the a principal inertial axis (see Table 1), and therefore the frequency scans were directed to search for the most intense μ_b transitions. Furthermore, to produce a further enhancement of the possible spectra, we used the ^{15}N sample for these scans, which is free of nuclear quadrupole coupling hyperfine effects. However, after long scans aimed to locate the $1_{1,1} \leftarrow 0_{0,0}$, $2_{1,2} \leftarrow 1_{0,1}$ and $3_{0,3} \leftarrow 2_{1,2}$ rotational transitions, no evidence was found of this conformer, using Ne or He as carrier gases. Conformer IIb has rotational parameters close to those of conformer IIa, and their spectra should appear in the same region. No lines attributable to this conformer were observed.

B. Structures. The experimental rotational constants of Tables 2–5 have been used to determine the r_s and r_0 structures

Table 4. Rotational Parameters for the Parent, ^{13}C , and ^{15}N Isotopomers of Conformer IIa of Alanine

	parent	$^{13}\text{C}_1$	$^{13}\text{C}_2$	$^{13}\text{C}_3$	^{15}N
A/MHz^a	4973.05578(61) ^d	4972.91787(64)	4962.6542(15)	4884.70481(92)	4918.67072(26)
B/MHz	3228.33792(45)	3215.88001(50)	3214.71567(79)	3188.97210(84)	3185.34089(20)
C/MHz	2307.8087(34)	2301.61912(40)	2302.42691(65)	2274.47477(75)	2275.83229(18)
Δ_J/kHz	2.125(14)	2.163(15)	2.089(26)	2.161(25)	2.1210(96)
Δ_{JK}/kHz	−4.842(58)	−5.017(63)	−4.635(90)	−4.643(93)	−5.200(20)
Δ_K/kHz	[4.982] ^e	[4.982]	[4.982]	[4.982]	4.982(23)
δ_J/kHz	0.412(13)	0.402(14)	0.387(23)	0.397(25)	0.4033(23)
δ_K/kHz	[7.349]	[7.349]	[7.349]	[7.349]	7.349(13)
χ_{aa}/MHz	0.4515(17)	0.4549(20)	0.4385(31)	0.3497(35)	
χ_{bb}/MHz	0.3267(21)	0.3268(30)	0.3298(42)	0.4961(49)	
χ_{cc}/MHz	−0.7782(21)	−0.7817(30)	−0.7683(42)	−0.8458(49)	
σ/kHz^b	1.6	1.6	2.0	2.6	0.7
N^c	38	32	22	30	17

^a Parameter definition as in Table 2. ^b rms deviation of the fit. ^c Number of transitions. ^d Standard error in parentheses in units of the last digit. ^e Parameters in square brackets were kept fixed in the fit.

Table 5. Rotational Parameters for the Deuterated Isotopomers of Conformer IIa of Alanine

	$\text{C}_2\text{-D}$	$\text{C}_3\text{-D}_3$	COO-D	N-D_a	N-D_b
A/MHz^a	4857.546(17) ^d	4531.58(14)	4834.8537(71)	4937.981(58)	4808.163(20)
B/MHz	3173.8738(11)	3030.166(36)	3220.2161(12)	3113.7956(28)	3150.7645(35)
C/MHz	2298.9205(11)	2159.054(16)	2280.62126(87)	2245.8672(26)	2263.7572(25)
Δ_J/kHz	2.070(56)	[2.121]	[2.121]	[2.121]	[2.121]
Δ_{JK}/kHz	[−5.20] ^e	[−5.20]	[−5.20]	[−5.20]	[−5.20]
Δ_K/kHz	[4.98]	[4.98]	[4.98]	[4.98]	[4.98]
δ_J/kHz	[0.403]	[0.403]	[0.403]	[0.403]	[0.403]
δ_K/kHz	[7.349]	[7.349]	[7.349]	[7.349]	[7.349]
χ_{aa}/MHz	0.395(7)	[0.4515]	[0.4515]	[0.4515]	[0.4515]
χ_{bb}/MHz	0.307(14)	[0.3267]	[0.3267]	[0.3267]	[0.3267]
χ_{cc}/MHz	−0.702(14)	[−0.7782]	[−0.7782]	[−0.7782]	[−0.7782]
σ/kHz^b	5.2	32.0	7.2	17.0	21.0
N^c	18	6	13	12	13

^a Parameter definition as in Table 2. ^b rms deviation of the fit. ^c Number of transitions. ^d Standard error in parentheses in units of the last digit. ^e Parameters in square brackets were kept fixed in the fit.

Table 6. Substitution r_s Coordinates (in Å) and Derived r_s Parameters for the Conformers I and IIa of Alanine (See Inserted Figures for Labeling and Principal Inertial Axis System Orientation)

Atom	Conformer I			Conformer IIa		
	<i>a</i>	<i>b</i>	<i>c</i>	<i>a</i>	<i>b</i>	<i>c</i>
C ₁	0.711(2) ^a	-0.10(1)	-0.09(1)	0.775(2)	0 ^b	-0.10(1)
C ₂	-0.712(2)	0.154(8)	-0.391(3)	-0.696(2)	0.177(7)	-0.430(3)
C ₃	-1.221(1)	1.2981(9)	0.534(2)	-1.2704(9)	1.269(1)	0.542(2)
N	-1.6134(7)	-0.964(1)	-0.309(4)	-1.4218(8)	-1.047(1)	-0.290(4)
H _α	-0.712(2)	1.158(8)	-1.5090(8)	-0.740(2)	0.532(2)	-1.4693(8)
H _β	2.4531(5)	0.520(2)	-0.177(7)	0.219(6)	-1.6004(8)	0.601(2)
H _a	-1.5815(8)	-1.2945(9)	0.720(2)	-2.3484(5)	-0.745(2)	0 ^c
H _b	-1.3107(9)	-1.6730(7)	-0.958(1)	-1.4883(8)	-1.4100(8)	-1.2753(9)

Parameter	r_s	r_s
$r(\text{C}_1\text{--C}_2)/\text{Å}$	1.48(1)	1.517(7)
$r(\text{C}_3\text{--C}_2)/\text{Å}$	1.57(1)	1.571(9)
$r(\text{N--C}_2)/\text{Å}$	1.438(9)	1.430(9)
$r(\text{H}_\alpha\text{--C}_2)/\text{Å}$	1.119(4)	1.100(6)
$r(\text{H}_\alpha\text{--N})/\text{Å}$	1.082(6)	1.017(4)
$r(\text{H}_\beta\text{--N})/\text{Å}$	1.008(6)	1.052(6)
$r(\text{H}_\alpha\cdots\text{H}_\beta)/\text{Å}$	1.742(4)	1.675(4)
$r(\text{H}_\alpha\cdots\text{N})/\text{Å}$	4.331(3)	1.947(9)
$\angle\text{C}_3\text{--C}_2\text{--C}_1/\text{deg}$	109(1)	107.6(8)
$\angle\text{N--C}_2\text{--C}_1/\text{deg}$	117(1)	111.8(7)
$\angle\text{H}_\alpha\text{--C}_2\text{--C}_1/\text{deg}$	102(1)	106.3(8)
$\angle\text{H}_\alpha\text{--N--C}_2/\text{deg}$	105.9(5)	103.7(5)
$\angle\text{H}_\beta\text{--N--C}_2/\text{deg}$	108.8(6)	103.6(5)
$\angle\text{H}_\alpha\text{--N--H}_\beta/\text{deg}$	112.8(4)	108.2(5)
$\angle\text{H}_\alpha\cdots\text{C}_1\text{--C}_2/\text{deg}$	147(2)	83.7(6)
$\angle\text{N--C}_2\text{--C}_1\text{--C}_3/\text{deg}$	-129(2)	-121.2(8)
$\angle\text{H}_\alpha\text{--C}_2\text{--C}_1\text{--C}_3/\text{deg}$	135(1)	118(1)
$\angle\text{H}_\alpha\text{--N--C}_2\text{--C}_1/\text{deg}$	62.(1)	148(2)
$\angle\text{H}_\beta\text{--N--C}_2\text{--C}_1/\text{deg}$	-59.4(1)	-99(1)
$\angle\text{N--C}_2\text{--C}_1\cdots\text{H}_\alpha/\text{deg}$	166(1)	-14(1)
$\angle\text{N--C}_2\text{--C}_1\text{--C}_3/\text{deg}$	-129(2)	-121.2(8)

^a Errors in parentheses in units of the last digit. These were calculated according to the formula of Costain³⁰ $\delta z_i = 0.0012/|z_i|$ Å. ^b Fixed to zero. Value calculated from the moments of inertia $|b| = 0.09(1)$ i. ^c Fixed to zero. The value calculated from the moments of inertia, $|c| = 0.483(3)$, gives rise to unrealistic structural parameters (see text).

of the observed conformers of alanine. The most direct structural analysis uses the Kraitchman's equations²⁹ to derive the squares of the *a*, *b*, and *c* coordinates of an isotopically substituted atom from the moments of inertia of the parent molecule and the corresponding singly substituted isotopomer. From the rotational parameters of Tables 2–5, the r_s structure of part of the skeleton of the observed conformers of alanine, including the three C atoms, the NH₂ group, and the carboxyl group hydrogen atom, can be obtained. The substitution r_s coordinates determined for both conformers are listed in Table 6. The absolute values of these coordinates are only consistent with plausible structures for conformers I and IIa of alanine. The sign ambiguities for larger coordinates were easily resolved by inspection, while for the small coordinates the signs chosen were those yielding the most reasonable r_s bond lengths and angles. It is well-known that the errors arising from those of the rotational constants are low if compared with possible errors arising from vibration–rotation contributions. For that reason the uncertainties, δz_i , in

Table 6 were calculated according to Costain formula:³⁰ $\delta z_i = 0.0012/|z_i|$ Å, which takes into account this fact and gives more realistic errors. The deficiencies of the r_s coordinates, due to zero-point vibrational effects, become apparent for atoms lying near the inertial axes. This is the reason for the imaginary *b* coordinate of atom C₁ of conformer IIa, which was set to zero. This is also the case of the H_a atom of conformer IIa for which the substitution coordinates were found to give unrealistic parameters. Inspection of any plausible structure of this conformer indicates that this atom is lying very close to the *ab* inertial plane, and therefore we decided to set its *c* coordinate to zero.

The r_s bond lengths and angles obtained from the center-of-mass coordinates are also given in Table 6. The parameters associated with the heavy atoms are the same for both conformers within 3 times the quoted errors, indicating the

(29) Kraitchman, *J. Am. J. Phys.* **1953**, *21*, 17.

(30) Van Eijck, B. P. *J. Mol. Spectrosc.* **1982**, *91*, 348.

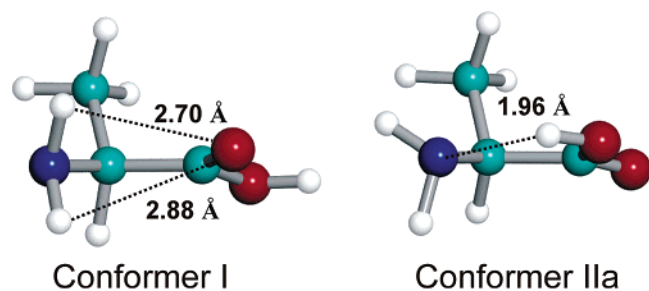


Figure 5. Structures of conformers I and IIa of alanine determined in this work, drawn to scale, showing the hydrogen bond distances.

consistency of the data. Despite the absence of information for the two oxygen atoms, the r_s parameters of Table 6 give the main details of the structure of the observed conformers of alanine. If a planar structure is assumed for the COOH group, the values of dihedral angle $\angle\text{N}-\text{C}_2-\text{C}_1\cdots\text{H}_\alpha$, $166(1)^\circ$ for conformer I and $-14(1)^\circ$ for conformer IIa suggest that the amino acid backbone is nonplanar in both conformers. The distance $r(\text{N}-\text{H}_\alpha) = 1.947(9)$ Å for conformer IIa is consistent with the formation of a $\text{O}-\text{H}\cdots\text{N}$ hydrogen bond. The formation of a nonsymmetrical bifurcated hydrogen bond of the type $\text{NH}\cdots\text{O}=\text{C}$ for conformer I can be also deduced from the distances $r(\text{H}_\alpha\cdots\text{O}_1) = 2.63$ Å and $r(\text{H}_\beta\cdots\text{O}_1) = 2.78$ Å, calculated assuming a planar structure of the COOH group equal to that determined for glycine^{7f} ($r(\text{C}=\text{O}_1) = 1.207$ Å, $r(\text{C}-\text{O}_2) = 1.357$ Å, $\angle\text{O}_1=\text{C}-\text{O}_2 = 123.5^\circ$). The values obtained for the nonbonding angle $\angle\text{H}_\alpha\cdots\text{C}_1-\text{C}_2$ allow us to conclude that the configuration of the carboxyl group is cis for conformer I and trans for conformer IIa.

Alternatively, all 30 moments of inertia of each conformer have been used to fit the effective ground-state r_0 structures shown in Figure 5 and Table 7 in an iterative least squares procedure as outlined by Schwendeman.³¹ The parameters associated with the H_α atom for both conformers and H_a atom for conformer IIa were found to be not determinable and were constrained to the corresponding r_s values. A local C_{3v} symmetry was considered for the CH_3 group for which the $r(\text{C}-\text{H})$ distances were fixed to 1.095 Å. The derived effective structures are consistent between them and with the previously described r_s structures. The general conclusions pointed out previously from the r_s results apply also to the r_0 structures of alanine.

C. Conformer Abundances. The absence of alanine IIb and type III conformers in the supersonic jet can be associated to collisional relaxation of these conformers to the most stable conformers IIa and I, respectively, through low-energy barriers.¹⁷ As an example, Figure 2 illustrates the MP2/6-311++G(d,p) energy profile calculated for the rotation of the $\angle\text{NCC}=\text{O}$ dihedral angle, which is consistent with this hypothesis. A low-energy barrier of 274 cm^{-1} is calculated between conformers IIIa and IIIb that may interconvert without cost of energy to conformer I. The same arguments were invoked to justify the nonobservation of the type III conformations in glycine¹⁷ and valine.⁵

In the present work, relative intensity measurements³² on different μ_a -type and μ_b -type transitions of conformers I and IIa using Ne as carrier gas have been done. These measurements

Table 7. Effective (r_0) Structure of Alanine (See Table 6 for Notation)

parameter	conformer I	conformer IIa
$r(\text{C}_1-\text{C}_2)/\text{Å}$	1.51(1) ^a	1.524(7)
$r(\text{C}_3-\text{C}_2)/\text{Å}$	1.53(2)	1.543(8)
$r(\text{N}-\text{C}_2)/\text{Å}$	1.45(1)	1.458(9)
$r(\text{O}=\text{C}_1)/\text{Å}$	1.24(2)	1.20(2)
$r(\text{O}-\text{C}_1)/\text{Å}$	1.33(2)	1.37(2)
$r(\text{H}_\alpha-\text{C}_2)/\text{Å}$	[1.119] ^b	[1.100] ^b
$r(\text{H}_\alpha-\text{N})/\text{Å}$	1.08(1)	[1.015] ^b
$r(\text{H}_\beta-\text{N})/\text{Å}$	1.01(1)	1.072(6)
$r(\text{H}_\alpha-\text{O})/\text{Å}$	0.99(3)	0.92(2)
$r(\text{H}-\text{C}_3)/\text{Å}$	[1.095] ^c	[1.095] ^c
$r(\text{H}_\alpha\cdots\text{H}_\beta)/\text{Å}$	1.74(2)	1.69(1)
$r(\text{H}_\alpha\cdots\text{N})/\text{Å}$	4.34(2)	1.96(1)
$r(\text{H}_\alpha\cdots\text{O})/\text{Å}$	2.70(2)	4.31(2)
$r(\text{H}_\beta\cdots\text{O})/\text{Å}$	2.88(2)	3.97(2)
$\angle\text{C}_3-\text{C}_2-\text{C}_1/\text{deg}$	108.3(6)	107.1(3)
$\angle\text{N}-\text{C}_2-\text{C}_1/\text{deg}$	115(1)	111.7(7)
$\angle\text{H}_\alpha-\text{C}_2-\text{C}_1/\text{deg}$	[102] ^b	[106.3] ^b
$\angle\text{H}_\alpha-\text{N}-\text{C}_2/\text{deg}$	105.6(7)	[103.6] ^b
$\angle\text{H}_\beta-\text{N}-\text{C}_2/\text{deg}$	109(1)	102.8(6)
$\angle\text{H}_\alpha-\text{N}-\text{H}_\beta/\text{deg}$	113(2)	108(1)
$\angle\text{H}_\alpha\cdots\text{C}_1-\text{C}_2/\text{deg}$	144(2)	84(1)
$\angle\text{C}_2-\text{C}_1=\text{O}/\text{deg}$	125(2)	125(1)
$\angle\text{C}_2-\text{C}_1-\text{O}/\text{deg}$	113(2)	113(1)
$\angle\text{H}-\text{C}_3-\text{C}_2/\text{deg}$	109.8(2)	[109.8] ^d
$\angle\text{N}-\text{C}_2-\text{C}_1-\text{C}_3/\text{deg}$	-123.5(7)	-121.1(7)
$\angle\text{H}_\alpha-\text{C}_2-\text{C}_1-\text{C}_3/\text{deg}$	[135] ^b	[118] ^b
$\angle\text{H}_\alpha-\text{N}-\text{C}_2-\text{C}_1/\text{deg}$	60.7(7)	[148] ^b
$\angle\text{H}_\beta-\text{N}-\text{C}_2-\text{C}_1/\text{deg}$	-61(1)	-99.2(4)
$\angle\text{N}-\text{C}_2-\text{C}_1\cdots\text{H}_\alpha/\text{deg}$	166(2)	-12(1)
$\angle\text{N}-\text{C}_2-\text{C}_1=\text{O}/\text{deg}$	-14(1)	167(1)
$\angle\text{N}-\text{C}_2-\text{C}_1-\text{O}/\text{deg}$	167(1)	-11.5(8)
$\angle\text{H}-\text{O}-\text{C}_1=\text{O}/\text{deg}$	-1(2)	-179(2)

^a Errors in parentheses in units of the last digit. ^b Fixed to the r_s values (see Table 6). ^c Assumed values. ^d Fixed to the r_0 value determined for conformer I.

can be related to the relative populations in the jet by assuming that the cooling in the supersonic expansion brings all the molecular systems to the lowest vibrational state of each observed conformer. The intensities of the μ_a -type or μ_b -type lines of conformer i , with number density N_i in the jet, have been assumed to be proportional to $(\mu_a)^i N_i$ or $(\mu_b)^i N_i$, respectively, where $(\mu_a)^i$ and $(\mu_b)^i$ are the projections of the electric dipole moment of conformer i along the a and b principal inertial axis. We used μ_{ai} instead of μ_{ai}^2 since the intensities depend on the amplitude of the free induction decay electric field. The obtained intensity ratios $\text{IIa/I} = 2.3(3)$, for μ_a -type transitions, and $\text{IIa/I} = 0.22(3)$, for μ_b -type transitions, give the same population ratio N_I/N_{IIa} after taking into account the experimental values of the electric dipole moment components.⁸ The total mean ratio $N_I/N_{\text{IIa}} = 3.7(5)$ indicates that conformer I is more stable than conformer IIa.

The observed relative abundances of conformers I and IIa of alanine in the supersonic expansion are the result of a series of processes that include the laser-vaporization of solid alanine, the seeding of alanine molecules in the region where the laser ablation plume and the carrier gas stream cross each other, and the collisional cooling occurring in the subsequent supersonic expansion. Laser ablation is a complex process that may result in high translational temperatures and collisionally induced vibration and rotational cooling.³³ The relative population of

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the different conformers of alanine would be brought close to that of thermodynamic equilibrium at the temperature of the carrier gas only if a high collision rate exists in the seeding region. During the expansion, collisional relaxation of high-energy conformers to low-energy conformers presumably occurs as discussed previously for conformers IIb and IIIa. In this way, the observed relative population ratio N_I/N_{IIa} can be tentatively related to the equilibrium distribution of conformers at the temperature of the carrier gas assuming that a high collisional rate exists in the seeding region. The hypothetical equilibrium relative populations of the lowest-energy conformers of alanine at 298 K have been calculated for the Gibbs energies, given in Table 1 to be $N_I/N_{IIa}/N_{IIb}/N_{IIIa} = 68:10:11:11$. Assuming that collisional relaxation brings the molecular systems in vibrational states of conformers I and IIIa to the lowest vibrational state of conformer I and that all molecules originally in conformers IIa and IIb finally occupy the lowest vibrational state in conformer IIa, a postexpansion ratio $N_I/N_{IIa} = 4$ is obtained. This ratio is in excellent agreement with that obtained from the experimental relative intensity measurements. This result seems to indicate that in our laser ablation experiment the collisional rate in the seeding region is high enough to bring the molecular system to a population distribution close to those of equilibrium at the temperature of the carrier gas.

Godfrey et al.,⁸ in their work on alanine, report a postexpansion population ratio $N_I/N_{IIa} = 8$ estimated from relative intensity measurements on the free-jet absorption millimeter-wave spectra. The discrepancy with the result given in this work is noteworthy, although comparison is difficult because the millimeter-wave data has no quoted error. In that experiment, alanine was vaporized by heating the sample at 225 °C and seeding it in a stream of Ar. In this case, the population of the different alanine conformers is brought to the equilibrium ratio in the pre-expansion mixture, and thus the relative population of conformers in the jet can be directly related to the pre-expansion equilibrium distribution. Using the theoretical free energies at 498.15 K given in Table 1, taking into consideration only conformers I, IIa, IIb, and IIIa, and assuming the relaxation of conformers IIIa to I and IIb to IIa in the expansion, we have estimated a postexpansion ratio of $N_I/N_{IIa} = 3/1$ for the free-jet experiment. This is also in reasonable agreement with our experimental ratio but far from that reported in the millimeter-wave work.

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Conclusions

In the present work we have investigated the rotational spectra of alanine using LA-MB-FTMW spectroscopy. The spectra of conformers I and IIa have been observed for the parent and different isotopic species under sub-Doppler resolution conditions. The experimental observations corroborate that conformer I is the most stable conformer of alanine.

The hyperfine structure of alanine conformers has been completely resolved, and the diagonal elements χ_{aa} , χ_{bb} , and χ_{cc} of the ^{14}N quadrupole coupling tensor have been determined. The notable agreement between the experimental and ab initio constants supports the assignments⁸ of conformers I and IIa and confirms that nuclear quadrupole coupling constants are very useful to discriminate the observed conformers of gas-phase amino acids.

The independent structures of conformers I and IIa of alanine have been determined experimentally for the first time. For conformer I (see Figure 5), the COOH group adopts a planar cis configuration, and a bifurcated hydrogen bond is formed between the amino group and carbonyl oxygen. Because of the nonplanarity of the amino acid backbone ($\angle\text{N}-\text{C}_2-\text{C}_1=\text{O} = -14^\circ$ (1)), this bifurcated hydrogen bond is unsymmetrical, as is revealed by the net difference between the distances $r(\text{N}-\text{H}_a\cdots\text{O}=\text{C}) = 2.70(2)$ Å and $r(\text{N}-\text{H}_b\cdots\text{O}=\text{C}) = 2.88(2)$ Å. For conformer IIa, the COOH group adopts a planar trans configuration, and a hydrogen bond is formed between the hydroxyl group and the amino group ($r(\text{O}-\text{H}\cdots\text{N}) = 1.96(2)$ Å). The amino acid backbone is also nonplanar ($\angle\text{N}-\text{C}_2-\text{C}_1=\text{O} = 168^\circ$) for this conformer.

The agreement between the observed and calculated relative conformer abundances in the supersonic expansion can be taken as a proof of the existence of a high collision rate in the region where the laser ablation plume and the carrier gas stream cross each other. Such a rate would bring the laser-vaporized molecules in the seeding region to a conformer distribution close to that of equilibrium at the temperature of the carrier gas.

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Supporting Information Available: Tables of experimental transition frequencies of all the observed isotopic species of conformers I and IIa of alanine (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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