J. Am. Chem. Soc. **1993,** *115,* 9687–9691

Millimeter-Wave Spectroscopy of Biomolecules: Alanine

P. D. Godfrey, S. Firth, L. D. Hatherley, R. D. Brown,[*] and A. P. Pierlot

Contribution from the Centre for High-Resolution Spectroscopy and Opto-Electronic Technology, Chemistry Department, Monash University, Clayton, Victoria 3168, Australia

Received January 26, 1993. Revised Manuscript Received May 28, 1993[•]

Abstract: We have observed the rotational spectrum of alanine using our Stark-modulated free-expansion jet spectrometer. Spectra arising from two different conformers have been identified and analyzed, yielding the following two sets of spectroscopic constants (MHz): (x) $A = 5066.1332(91)$, $B = 3100.9368(66)$, $C = 2264.0428(92)$; (y) $A = 4973.0600(64)$, $B = 3228.3220(99)$, $C = 2307.821(15)$. Dipole moment components have also been determined from the Stark effect. Species x has been identified with conformer I and y with conformer III (see Figure 2). These are the two conformers corresponding to those previously identified for glycine. We estimate their relative abundances in the expanding plume to be about 8:1 in favor of I.

Introduction

The study of the rotational spectra of amino acids, as of most other biomolecules, has proved a challenge because of the difficulty of vaporizing them without decomposition. The early studies of glycine[1,2] required some instrumental developments to enable the spectrum to be observed. With the more recent development of a Stark-modulated expanding jet spectrometer,[3] it is now possible to observe such spectra more readily, opening the possibility of further studies of larger amino acids than glycine. As part of our continuing program of studying biomolecules,[4] we report here a study of the rotational spectrum of alanine.

In the crystal alanine exists as a zwitterion.[5] As in the case of the simpler amino acid glycine,[6] the crystal contains zwitterions connected by a network of hydrogen bonds.[5d] In the case of structural studies of alanine in the gas phase, low-resolution photoelectron spectroscopy[7] did not yield any conformational information. An electron diffraction study[8] indicated that there was only one conformer present. However only the heavy-atom positions were established in this study, the electron diffraction patterns being too insensitive to hydrogen positions to enable the hydrogens to be located. Therefore reliance was placed on molecular orbital calculations to decide the nature of the conformer.

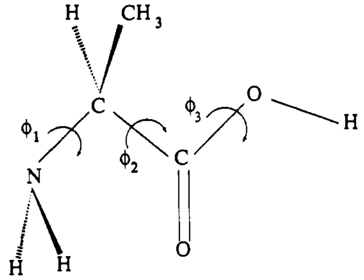

Figure 1. Reference conformation ($\phi_1 = \phi_2 = \phi_3 = 0$) and definition of torsional angles for alanine conformers, where ϕ_1 is the dihedral angle between the bisector of the HNH angle and the C–C bond involving the carboxyl group.

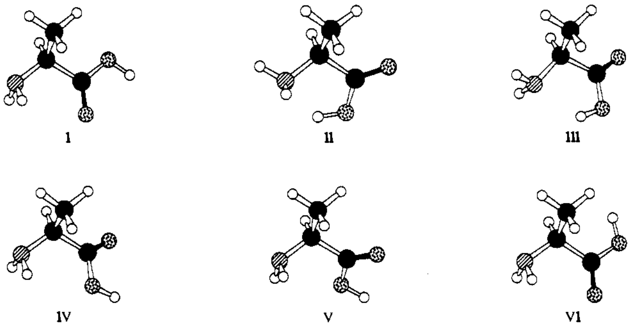

Figure 2. Most stable conformers of alanine.

Although there have been numerous *ab initio* calculations performed on glycine,[9] there have been few investigations of the potential energy surface of alanine. Sellers and Schäfer[10] examined the two alanine conformers corresponding to the conformers of glycine observed thus far, using a 4-21G basis set. Siam et al,[11] at the same level of theory, examined the N—C—C═O (φ_2) torsional potential energy curve near $\varphi_1 = 0°$ and $\varphi_3 = 0°$ (these geometric parameters are defined in Figure 1). Minima were found to occur at $\varphi_2 = 0°, 150°$, and $-150°$. All conformers predicted by these molecular calculations, for both glycine and alanine, involve stabilization through intramolecular hydrogen bonding.

We report here the rotational spectra of two conformers of alanine together with molecular orbital calculations using a larger basis set (6-31G**).

Theoretical Procedure

Theoretical calculations were carried out using the GAUSSIAN 90 package[12] at the HF/6-31G** level. Six conformers (see Figure 2) were found on the alanine potential energy

---

[•] Abstract published in *Advance ACS Abstracts*, September 1, 1993.

[1] Brown, R. D.; Godfrey, P. D.; Storey, J. W. V.; Bassez, M. P. *J. Chem. Soc., Chem. Commun.* **1978**, 547.

[2] Suenram, R. D.; Lovas, F. J. *J. Mol. Spectrosc.* **1978**, *72*, 372; *J. Am. Chem. Soc.*, **1980**, *102*, 7180–7184.

[3] Brown, R. D.; Crofts, J G.; Godfrey, P. D.; McNaughton, D.; Pierlot, A. P. *J. Mol. Struct.* **1988**, *190*, 185–193.

[4] Vogelsanger, B.; Godfrey, P. D.; Brown, R. D. *J. Am. Chem. Soc.* **1991**, *113*, 7864–7869 and references cited therein.

[5] (a) Levy, H. A.; Corey, R. B. *J. Am. Chem. Soc.* **1941**, *63*, 2095. (b) Donohue, J. *J. Am. Chem. Soc.* **1950**, *72*, 949. (c) Simpson, H. J., Jr.; Marsh, R. E. *Acta Crystallogr.*, **1966**, *20*, 550. (d) Lehmann, M. S.; Koetzle, T. F.; Hamilton, W. C. *J. Am. Chem. Soc.* **1972**, *94*, 2657.

[6] Marsh, R. E. *Acta Crystallogr.* **1958**, *11*, 654.

[7] (a) Debies, T. P.; Rabalais, J. W. *J. Electron Spectrosc. Relat. Phenom.* **1974**, *3*, 315. (b) Klasinc, L. *J. Electron Spectrosc. Rel. Phen.* **1976**, *8*, 161.

[8] Iijima, K.; Beagley, B. *J. Mol. Struct.* **1991**, *248*, 133.

[9] (a) Vishveshwara, S.; Pople, J. A. *J. Am. Chem. Soc.* **1977**, *99*, 2422. (b) Sellers, H. L.; Schäfer, L. *J. Am. Chem. Soc.* **1978**, *100*, 7728. (c) Ramek, M.; Cheng, V. K. W.; Frey, R. F.; Newton, S. Q.; Schäfer, L. *J. Mol. Struct.* **1991**, *235*, 1. (d) Jensen, J. H.; Gordon, M. S. *J. Am. Chem. Soc.* **1991**, *113*, 7917. (e) Frey, R. F.; Coffin, J.; Newton, S. Q.; Ramek, M.; Cheng, V. K. W.; Schäfer, L. *J. Am. Chem. Soc.* **1992**, *114*, 5369. (f) Hu, C. H.; Shen, M.; Schaefer, H. F. *J. Am. Chem. Soc.*, submitted.

[10] Sellers, H. L.; Schäfer, L. *Chem. Phys. Lett.* **1979**, *63*, 609.

[11] Siam, K.; Klimkowski, V. J.; Ewbank, J. D.; Van Alsenoy, C.; Schafer, L. *J. Mol. Struct. (THEOCHEM)* **1984**, *110*, 171.

0002-7863/93/1515-9687$04.00/0

Table I. Molecular Parameters Predicted by *Ab Initio* Molecular Orbital Calculations[a]

	alanine I	alanine II	alanine III	alanine IV	alanine V	alanine VI
φ_1	–1.6	147.0	214.3	–1.6	4.6	–3.1
φ_2	–11.5	199.4	161.6	143.3	–131.2	–14.7
φ_3	–0.6	177.1	182.0	0.5	–0.1	178.1
A/MHz	5145.1	4970.3	5073.1	5137.8	5135.3	5086.2
B/MHz	3169.8	3474.4	3191.4	2931.5	3297.9	3157.7
C/MHz	2269.1	2173.2	2377.0	2468.9	2184.8	2289.5
Δ/u Å^2	–34.9	–14.6	–45.4	–66.1	–20.3	–38.7
E_{rel}/kJ mol^{-1}	0	9.9	10.8	6.3	6.8	29.6
μ_a/D	0.8	5.0	5.4	0.1	0.7	1.6
μ_b/D	1.1	2.2	1.3	1.4	1.4	2.7
μ_c/D	0.3	0.3	0.6	1.1	0.1	1.6
μ_{tot}/D	1.4	5.5	5.5	1.8	1.6	3.5
χ_{aa}/MHz	–3.2	–1.2	0.9	–3.4	–3.4	–3.1
χ_{bb}/MHz	1.7	–0.6	0.4	1.8	2.0	1.7

[a] All calculated using the 6-31G** basis set.

surface: I, the global minimum which corresponds to that found for glycine, two sets of pairs (II/III and IV/V) which arise as a result of the asymmetry of alanine, and VI, which is computed to be the least stable of the six. Their dihedral angles and associated parameters are listed in Table I.

The theoretical values of the rotational constants show that it is not feasible to distinguish unequivocally between the six conformers purely from the experimentally determined rotational constants. The rotational constants of conformers I and VI in particular are very close. However their dipole moments differ considerably. Within each pair of conformers II/III and IV/V, distinguishing parameters that set them apart are the differences in the B and C rotational constants and in their inertial defects ($\Delta = I_c - I_a - I_b$). Conformers II and III differ from the remaining conformers and from each other in their hyperfine coupling constants.

At the level of theory used, the conformer pair IV/V is predicted to be more stable than the pair II/III. Hu et al,[9f] in their *ab initio* work on glycine, found that the self-consistent field (SCF) and single and double excitation configuration interaction (CISD) methods gave results to the same effect. When the single and double excitation coupled cluster (CCSD) and the single, double, and perturbative triple excitation coupled cluster [CCSD(T)] methods were applied, the relative stabilities were reversed. It seems likely that a similar result might hold for alanine at these levels of theory. Conformers II and III have much greater dipole moments than IV and V and so have greater potentials to be observed in a pure rotational spectrum when at the same

Table II. Observed Transitions (MHz) of the Two Assigned Conformers of Alanine[a]

transition							alanine I[b]		alanine III[b]	
J'	K_{-1}'	K_1'		J''	K_{-1}''	K_1''	obsd freq	O–C[c]	obsd freq	O–C[c]
6	6	0	–	5	5	1	58 445.214(53)	–0.002	57 517.98(68)	–0.297
6	6	1	–	5	5	0	58 444.535(36)	0.009	57 517.09(38)	0.374
7	4	3	–	6	3	4	55 790.145(38)	0.047	56 573.952(35)	–0.012
7	4	4	–	6	3	3			52 155.968(32)	–0.000
7	5	2	–	6	4	3	59 252.93(24)	0.058	58 984.058(26)	–0.001
7	5	3	–	6	4	2	59 006.64(63)	0.076	58 528.514(32)	–0.010
7	6	1	–	6	5	1	63 872.63(14)	0.036		
7	6	1	–	6	5	2	63 879.314(49)	0.054	63 148.280(42)	0.093
7	6	2	–	6	5	1	63 871.627(52)	–0.039	63 131.048(41)	–0.079
7	6	2	–	6	5	2	63 878.294(72)	–0.042		
8	4	4	–	7	3	4	58 318.148(35)	–0.070		
8	4	4	–	7	3	5	62 990.230(28)	–0.020	64 791.236(26)	0.005
8	4	5	–	7	3	4			55 654.668(22)	0.004
8	4	5	–	7	3	5	61 153.986(36)	0.057		
8	5	3	–	7	4	4	64 888.441(49)	0.043	65 021.526(21)	0.004
8	5	4	–	7	4	3			63 481.774(35)	–0.004
8	6	2	–	7	5	3	69 309.285(37)	–0.018	68 785.663(46)	0.135
8	6	3	–	7	5	2	69 264.323(39)	–0.016	68 685.523(38)	–0.100
9	3	6	–	8	2	6	62 693.563(31)	–0.019		
9	4	5	–	8	3	6	71 452.839(29)	–0.002		
9	5	4	–	8	4	5	70 884.906(37)	0.017		
9	5	4	–	8	5	3			52 176.13(38)	0.109
9	5	5	–	8	5	4			51 473.15(35)	0.198
10	5	5	–	9	5	4			58 800.78(13)	–0.009
10	5	6	–	9	5	5			57 252.06(14)	0.014
10	7	3	–	9	7	2			56 871.262(61)	–0.142
10	7	4	–	9	7	3			56 858.86(18)	0.206
10	8	3	–	9	8	2			56 615.08(15)	0.290
10	9	2	–	9	9	1			56 449.193(65)	0.020
11	1	10	–	10	1	9	54 436.559(31)	–0.021	55 489.45(35)	0.087
11	1	11	–	10	1	10			52 333.85(23)	0.274
11	1	10	–	10	2	9	54 391.968(36)	–0.001	55 473.254(27)	0.012
11	2	10	–	10	1	9	54 453.750(28)	–0.012	55 494.830(24)	–0.013
11	2	9	–	10	2	8	57 811.436(67)	0.022	58 803.230(66)	0.007
11	2	10	–	10	2	9	54 409.146(70)	–0.006	55 478.79(19)	0.068
11	4	7	–	10	4	6	63 772.001(71)	0.044	65 984.027(75)	–0.027
11	4	8	–	10	4	7	59 755.075(29)	0.005	61 331.258(45)	0.009
11	5	6	–	10	5	5	62 483.743(45)	0.001	65 601.79(36)	0.154
11	5	7	–	10	5	6	60 798.597(29)	0.008	62 885.58(40)	0.135
11	6	5	–	10	6	4	61 007.37(10)	–0.024	63 681.426(70)	0.025
11	6	6	–	10	6	5	60 762.353(51)	0.009	63 109.059(77)	0.021
11	7	4	–	10	7	3	60 464.33(43)	–0.005	62 827.026(60)	–0.106
11	7	5	–	10	7	4	60 447.47(40)	0.028	62 776.851(83)	0.174
12	0	12	–	11	1	11	55 862.403(53)	0.146	56 947.11(21)	–0.039
13	1	13	–	12	0	12	60 388.153(49)	–0.066	61 560.17(21)	–0.093

[a] Numbers in parentheses are an estimate of the standard deviation in units of the least significant digit quoted, used for weighting purposes (see text). [b] Based on the assignment of the observed speces x to alanine I and y to alanine III. [c] Calculated using the least-squares fit rotational and centrifugal distortion constants given in Table III.

Table III. Experimentally Derived Spectroscopic Parameters of Alanine[a]

	alanine I[b]	alanine III[b]
A/MHz	5066.1332(91)	4973.0600(64)
B/MHz	3100.9368(66)	3228.3220(99)
C/MHz	2264.0428(92)	2307.821(15)
D_J/kHz	1.567(34)	1.411(49)
D_{JK}/kHz	−1.064(80)	−0.335(57)
D_K/kHz	0.87(13)	0.84(19)
d_1/kHz	−0.5706(71)	−0.408(10)
d_2/kHz	−0.4463(51)	−0.3954(96)
no. of lines in fit	33	38
RMS error in fit/kHz	43	131
Δ/u Å^2	−39.513	−39.183
rel beam concn/%	89	11
E_{rel}/kJ mol^{-1}	0	8.7(5)
μ_a/D[c]	0.6216(46)	4.924(14)
μ_b/D[c]	1.60(46)	1.4(2)[d]
μ_c/D[c]	0.3386(33)	0.2790(22)
μ_{tot}/D	1.8(5)	5.13(5)

[a] Numbers shown in parentheses refer to one standard deviation, in units of the least significant digit quoted, as calculated from least-squares fitting procedures. Two exceptions are for alanine III, μ_b as noted and for E_{rel}, where the standard deviation has been estimated from propagation of measurement uncertainties. [b] Based on the assignment of the observed species x to alanine I and y to alanine III. [c] From Stark-effect measurements given in Table IV. [d] From measured relative intensities of $9_{2,7}-8_{2,6}$ and $9_{3,7}-8_{2,6}$ transitions. Uncertainties were difficult to quantify because of line shape variations.

concentration as conformers IV and V. The latter might well be detected in deeper, more sensitive searches in the gas phase.

Experimental Section

The Stark-modulated supersonic jet spectrometer has been previously described.[3] Solid samples of alanine, obtained from Koch Light Laboratories Ltd., were vaporized at 235 °C in a stream of argon at about 30 kPa and further heated to about 255 °C before expansion. A number of strong lines were observed in the range 52–72 GHz. Their frequencies are listed in Table II, together with estimated uncertainties ($^1/_{10}$ line widths for resolved strong lines, proportionately greater uncertainties for weak and/or partly resolved lines) which were used as relative weightings in the least-squares fitting. The small incidence of observed–calculated values exceeding the estimated uncertainty (see Table II) indicates that in reality our estimated uncertainties are somewhat larger than one standard deviation.

Results and Discussion

The observed spectrum was relatively sparse, as is typical in free-expansion jet-cooled rotational spectroscopy. It proved possible to assign lines to two distinct spectroscopic species, hereafter referred to as x and y, using the Watson S-reduced Hamiltonian. In the region covered by our survey scans (54.2–65.4 GHz), all of the strongest lines (typically observed with S/N around 20 for a detection time constant of 1 s) and about 80% of all detected lines could be assigned to one or the other of the two species. A number of weak lines remained unassigned although we sought among them for further assignments of species representing other conformers of alanine. They had $S/N \leq 3$ apart from a single somewhat stronger line of S/N about 7.

From Stark-effect measurements on the $7_{6,2}-6_{5,2}$ and $12_{1,12}-11_{1,11}$ for one of the assigned species (x) and the $8_{5,3}-7_{4,4}$ and $11_{3,9}-10_{3,8}$ transitions for the other (y), we were able to obtain approximate values of the dipole moment components, as summarized in Table III. Dipole moment component values were least-squares fitted directly to the measured Stark-effect frequencies listed in Table IV via a computer program that solves the Stark-rotational Hamiltonian variationally and thus, unlike

Table IV. Stark-Effect Measurements Used in the Fitting of the Electric Dipole Moment Components of Alanine

bias voltage[b]	M_J	obsd freq/MHz[c]	O–C[d]	bias voltage[b]	M_J	obsd freq/MHz[c]	O–C[d]
(a) Alanine I Stark Measurements: $7_{6,2}-6_{5,2}$ (Calculated Zero-Field Frequency[a] = 63 878.338 MHz)							
0.0	6	63 878.324(45)	−0.004	1400.0	2	63 877.95(16)	−0.003
1000.0	1	63 876.90(18)	0.000	1400.0	3	63 879.03(10)	0.034
1000.0	2	63 877.41(39)	0.011	1400.0	5	63 881.15(30)	−0.010
1000.0	3	63 878.09(26)	−0.004	1600.0	1	63 877.18(9)	0.019
1000.0	4	63 878.82(41)	−0.021	1600.0	2	63 878.25(31)	0.004
1000.0	6	63 880.39(19)	0.011	1600.0	4	63 880.69(26)	−0.005
1200.0	1	63 876.98(24)	0.016	1600.0	5	63 881.94(24)	0.000
1200.0	2	63 877.70(43)	0.032	1800.0	1	63 877.28(8)	−0.001
1200.0	3	63 878.51(10)	−0.030	1800.0	2	63 878.53(20)	−0.016
1200.0	4	63 879.28(30)	−0.173	1800.0	4	63 881.28(28)	−0.040
1200.0	6	63 881.32(8)	0.006	1800.0	5	63 882.72(11)	0.000
1400.0	1	63 877.06(9)	0.006				
$12_{1,12}-11_{1,11}$ (Calculated Zero-Field Frequency[a] = 55 862.366 MHz)							
0.0	11	55 862.403(53)	0.011	3400.0	1	55 861.67(22)	−0.099
3000.0	3	55 860.418(68)	−0.010	3400.0	3	55 860.10(19)	−0.064
3000.0	5	55 859.016(42)	−0.007	3400.0	5	55 858.613(77)	0.037
(b) Alanine III Stark Measurements: $8_{5,3}-7_{4,4}$ (Calculated Zero-Field Frequency[a] = 65 021.519 MHz)							
0.0	7	65 021.526(21)	0.000	500.0	1	65 022.77(8)	−0.013
300.0	1	65 022.00(15)	0.021	600.0	1	65 023.34(7)	0.006
300.0	2	65 023.35(6)	0.008	700.0	1	65 023.98(37)	−0.004
300.0	3	65 025.58(69)	−0.017	800.0	1	65 024.80(16)	0.068
400.0	0	65 021.52(12)	−0.001	3600.0	0	65 021.17(7)	0.011
400.0	1	65 022.32(4)	−0.011	4000.0	0	65 021.07(9)	−0.003
400.0	2	65 024.69(18)	−0.055	5000.0	0	65 020.82(4)	0.002
$11_{3,9}-10_{3,8}$ (Calculated Zero-Field Frequency[a] = 58 600.174 MHz)							
0.0	10	58 600.08(11)	−0.006	4400.0	4	58 605.18(45)	−0.047
4000.0	2	58 601.04(52)	0.010	4600.0	2	58 601.37(21)	0.046
4400.0	2	58 601.22(11)	−0.002	5000.0	2	58 601.52(38)	−0.021

[a] Calculated using the least-squares fit rotational and centrifugal distortion constants given in Table III. [b] DC bias voltage to which square wave modulation voltage was clamped. For all measurements the electrode spacing was 4.477 cm. This was calibrated from the measured Stark effect of the $4_{1,3}-4_{0,4}$ transition of SO_2 by using the dipole moment μ_b = 1.633 05(4) D (Lovas, F. J. *J. Phys. Chem. Ref. Data* **1985**, *14*, 395–488.) [c] Numbers in parentheses are an estimate of the standard deviation in units of the least significant digit quoted, used for weighting purposes (see text). [d] Residuals from a direct nonlinear least-squares fit to the dipole moment components and the zero-field frequency. The best fit dipole moment component values are given with their uncertainties in Table III (see text).

more elementary treatments, is able to accomodate transitions involving near degeneracies that give rise to nonquadratic Stark shifts. For comparison, Table I lists dipole moment component values derived from molecular orbital calculations. For the species x, the experimentally derived rotational constants were within 2% of the corresponding theoretical values for alanine I or alanine VI but not the other four conformers. However the observed dipole moment components (see below) pointed unambiguously to an identification with alanine I. For the species y, the observed rotational constants were within 3% or better of those predicted for alanine III, those for alanine VI being almost as satisfactory. But again the dipole moment components clearly pointed to alanine III as the correct identification. The calculated dipole moment components of alanine II are similar to those of alanine III. The rotational constants *B* and *C* of alanine II, however, vary by 7.6% and 5.8%, respectively, from those of y.

The lines that showed ^{14}N nuclear quadrupole hyperfine structure helped to confirm the identities of the species x and y as the conformers I and III, respectively. In the case of the species x, a number of lines showed patterns closely similar to those predicted from the theoretical coupling constants of conformer I. Similar patterns would be expected for conformers IV, V, and VI but not for conformers II or III so that only partial identification is derivable from the hyperfine structure in this instance. Figure 3 shows traces of the $7_{4,4}$–$6_{3,3}$ transition for x and y together with those predicted for conformers I and III. For alanine III only a few very weak transitions were predicted to have a hyperfine pattern sufficiently resolvable for identification purposes. Of these, the $5_{3,3}$–$4_{0,4}$ transition of species y was observed (see Figure 4) and the splitting pattern confirmed that it is alanine III and not alanine II that can be identified as y.

We were not able to observe any line splittings that could be attributed to internal rotation of the methyl group in alanine. In the analogous cases of fluoropropionic acid[13] and lactic acid,[14] no splittings attributable to internal rotation of the methyl group were observed for the $v = 0$ and 1 states of the internal rotation. We detected only the ground vibrational state of alanine in our jet spectrometer, where we expect the cooling to depopulate excited low-frequency vibrational states, as in our recent study of 2-hydroxypyridine.[15] Since we predict a barrier height of about 1400 cm^{-1} (6-31G** basis set) for alanine, slightly greater than the experimentally derived barrier heights of 1130 cm^{-1} and 1000–1300 cm^{-1} for fluoropropionic and lactic acids, respectively, we would not expect to observe internal rotation splittings in alanine.

The degree of agreement between observed and calculated dipole moment components is similar to that found for some related compounds, i.e. discrepancies of up to 30% in the components and 20% in the total moments. It should perhaps be noted that, as in the case of glycine, the measured values of the dipole moments of the conformers rule out the possibility that alanine is in the zwitterionic form in the vapor phase although this is the tautomer that prevails in the crystalline state.

From the measured dipole moment components for the two conformers and the observed integrated areas of the strongest lines of the conformers, we can estimate the relative abundances as about 8:1 in favor of alanine I. Assuming that this ratio was "frozen in" at the expansion jet temperature of about 500 K, we estimate the difference in energies of the two conformers is about 9 kJ mol^{-1} in favor of alanine I. The *a*-type transitions of conformers I and III were observed to have similar absorption signal strengths but the ratio of the $\mu_a{}^2$ is 1:63, which is the dominant factor in making alanine III intrinsically much easier to detect, a situation similar to that found previously for glycine.[1,2] Bearing in mind that the strongest lines of alanine I were detected under search scan conditions with a $S/N \approx 20$, then it is possible, using the value of the largest *ab initio* principal axis dipole moment component for each conformer, to deduce approximate upper limits to the abundances (relative to those of I) of the unobserved alanine conformers in our jet. These are as follows: 1% for II, 13% for both IV and V, and 4% for VI. From these values it follows that the corresponding experimentally determined approximate lower limits for conformational energies relative to those of alanine I are 19 kJ mol^{-1} for II, 8 kJ mol^{-1} for IV and V, and 14 kJ mol^{-1} for VI. With the exception of II, these values are consistent with the relative energies predicted by molecular orbital calculations (Table I). Conversely, by accepting the *ab initio* values of dipole moments and relative energies and the observed sensitivity for conformers I and III, it follows that conformer II should have been detected in our search scan although it clearly was not found. However we note that the extensive studies of predicted relative energies of glycine conformers[9f] show that *ab initio* relative energies change when configuration interaction is incorporated and so the present experimental observations probably serve to emphasise that predictions at the HF/6-31G** level are not entirely reliable for predicting small energy differences between conformers, even if they provide a useful general guide as to which conformers need to be taken into consideration.

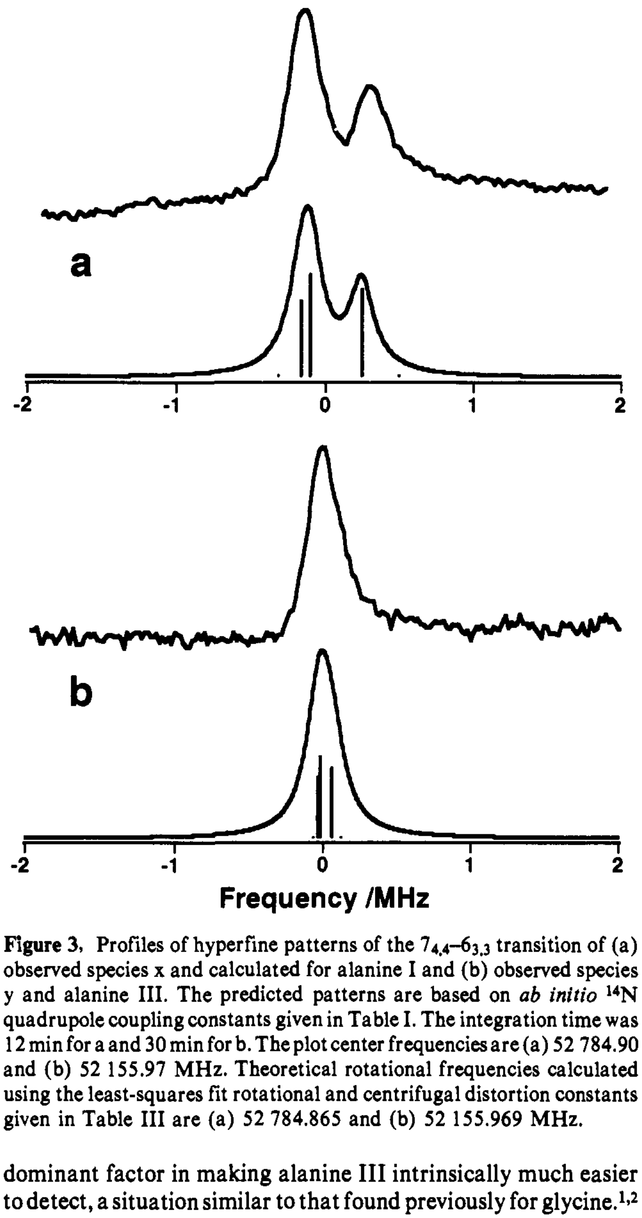

Figure 3. Profiles of hyperfine patterns of the $7_{4,4}$–$6_{3,3}$ transition of (a) observed species x and calculated for alanine I and (b) observed species y and alanine III. The predicted patterns are based on *ab initio* ^{14}N quadrupole coupling constants given in Table I. The integration time was 12 min for a and 30 min for b. The plot center frequencies are (a) 52 784.90 and (b) 52 155.97 MHz. Theoretical rotational frequencies calculated using the least-squares fit rotational and centrifugal distortion constants given in Table III are (a) 52 784.865 and (b) 52 155.969 MHz.

---

(12) Frisch, M. J.; Head-Gordon, M.; Trucks, G. W.; Foresman, J. B.; Schlegel, H. B.; Raghavachari, K.; Robb, M.; Binkley, J. S.; Gonzalez, C.; Defrees, D. J.; Fox, D. J.; Whiteside, R. A.; Seeger, R.; Melius, C. F.; Baker, J.; Martin, R. L.; Kahn, L. R.; Stewart, J. J. P.; Topiol, S.; Pople, J. A. *Gaussian 90, Revision I*; Gaussian, Inc.: Pittsburgh, PA, 1990.

(13) van Zoeren, E.; van Eijck, B. P. *J. Mol. Spectrosc.* **1984**, *103*, 75–86.

(14) van Eijck, B. P. *J. Mol. Spectrosc.* **1983**, *101*, 133–138.

(15) Hatherley, L. D.; Brown, R. D.; Godfrey, P. D.; Pierlot, A. P.; Caminati, W.; Damiani, D.; Melandri, S.; Favero, L. V. *J. Phys. Chem.* **1993**, *97*, 46–51.

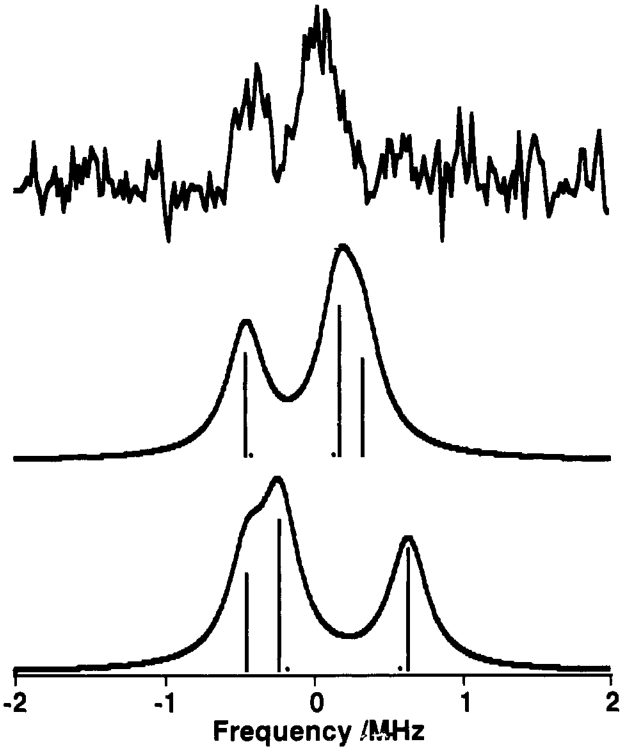

Figure 4. Profiles of hyperfine patterns of the $5_{3,3}$–$4_{0,4}$ transition: top, observed species y; middle, calculated for alanine III; bottom, alanine II. The integration time was 500 min. The observed plot center frequency is 51 843.80 MHz. Using the least-squares fit rotational and centrifugal distortion constants given in Table III, the theoretical rotational frequency for the alanine III $5_{3,3}$–$4_{0,4}$ transition is 51 843.716 MHz.

As in the case of glycine, the most stable conformers of alanine are stabilized by internal hydrogen bonding between the two amino hydrogens and the carboxyl oxygen (conformer I) or between the hydrogen of the carboxyl group and the lone pair of the amino nitrogen. This adds to a growing list of cases in which the favored conformers/tautomers of biologically important molecules are stabilized by angular hydrogen bond interactions between oxygen and nitrogen, or even, in a few cases, between NH and an aromatic (or heterocyclic) sextet.[16]

In their electron diffraction study of alanine, Iijima and Beagley[8] located the heavy atoms, finding that the amino N was *syn* to the carbonyl oxygen of the carboxyl group. Although they were unable to locate hydrogen atoms, they assumed that only one conformer was present in their vapor and that it was conformer I by analogy with glycine. They attributed the apparent lowering of stability of other conformers (such as conformer III) to adverse interactions involving the methyl group. From our results for the relative abundances of the two conformers, assuming that the conformational equilibrium was frozen at the jet temperature of about 500 K, we would expect their vapor to contain something like 11% of conformer III, which presumably is too low to noticeably affect their radial distribution curve.

Conclusion

Rotational constants for two species have been derived from analysis of the rotational spectrum of alanine vapor in a free-expansion jet. Stark-effect measurements have yielded dipole moment components. From comparison of both sets of constants with those predicted from *ab initio* molecular orbital calculations at the HF/6-31G** level, we have been able unambiguously to identify the two observed conformers as alanine I and alanine III (see Figure 2). These are the conformers analogous to those previously shown to be the two most stable gas-phase conformers of glycine. Some confirmation of the identifications is also provided by comparing some line profiles with those expected on the basis of theoretically predicted quadrupole hyperfine coupling constants. The relative concentrations of the two conformers in the jet are estimated to be I:III = 8:1.

Acknowledgment. This work was supported by a grant from the Australian Research Council.

---

(16) See, for example, the case of histamine, ref 4.