Rotational Spectrum of a Neurohormone: β-Alanine

Shane J. McGlone and Peter D. Godfrey*

Contribution from the Centre for High-Resolution Spectroscopy & Optoelectronic Technology, Chemistry Department, Monash University, Clayton, Victoria 3168, Australia

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Abstract: We have observed and assigned the rotational spectrum of β-alanine using our Stark-modulated free-expansion jet spectrometer. Two conformers of β-alanine have been identified, and they involve the same types of intramolecular interactions found in both gaseous glycine and α-alanine. Identification was based on a comparison of the results from parallel *ab initio* HF/6-31G** molecular orbital calculations and the observed rotational constants for the normal and N,N,O-d_3 isotopomers and ^{14}N quadrupole hyperfine multiplets. No evidence of a third conformer was observed despite the *ab initio* molecular orbital calculations predicting several energetically favorable conformations.

Introduction

The elucidation of the primary structural requirements for molecules to exhibit neuropharmacological activity is an area of vigorous research. It is found that many endogenous mammalian neurotransmitters, including those most important in the central nervous system—serotonin, dopamine, γ-aminobutyric acid (GABA), and acetylcholine—are surprisingly simple molecules based on an ethylamine substructure. The conformational preferences of this structural feature are believed to underlie the molecular recognition by which these various neurotransmitters act to affect the conductivity of ion channels associated with particular receptor sites.[1]

Glycine, β-alanine, and GABA are consecutive members of a homologous series of aminoacids; glycine and GABA are firmly established as central nervous system (CNS) inhibitory neurotransmitters. Distinct receptor sites for glycine and GABA have been found, and the structural similarities of β-alanine to each of these molecules enables it to bind at the receptor sites of both.[2] In contrast to other neurologically active aminoacids the concentration of β-alanine in nervous tissue is low,[3] and from its broader distribution is probably more correctly designated a neurohormone rather than a neurotransmitter.[2] However, along with aspartate, glutamate, and GABA, β-alanine is implicated as an actual neurotransmitter in the optic nerve of the mammalian visual system.[4]

It is widely believed that the solvated zwitterionic form of these aminoacids, not presently amenable to gas-phase structural investigation, interacts with the neuroreceptors. A sensitive test of the reliability of current theoretical methods for modeling this class of molecules is afforded by experimental structural studies on the corresponding nonzwitterionic tautomers. In an attempt to further explore the intramolecular interactions of amino acids we have analyzed the rotational spectrum of gaseous β-alanine, isolated from environmental effects, and furthered the *ab initio* molecular orbital calculations to the HF/6-31G** level of theory.

Previous Work

It has been shown from X-ray crystallographic studies[5] that in the solid state the zwitterionic form of β-alanine adopts a *gauche* conformation about the $C_{(2)}-C_{(3)}$ bond (see Figure 1). NMR studies[6] reveal that in aqueous solutions the zwitterion is distributed between *gauche* and *trans* conformations in the molar ratio 2:1 which, surprisingly, is independent of pH. The gas-phase conformers of glycine[7] and alanine[8] (i.e., α-alanine) have been identified from a comparison of the rotational constants and electronic properties determined in microwave spectroscopic investigations with the results from *ab initio* molecular orbital calculations. This general approach has been successful in our investigations by microwave spectroscopy of the conformers/tautomers of numerous molecules of biological significance.[9]

While the conformational possibilities of the β-alanine molecule have been determined *ab initio* in a complete survey of the potential energy surface at the HF/4-31G level of approximation,[10] similar calculations for glycine and alanine

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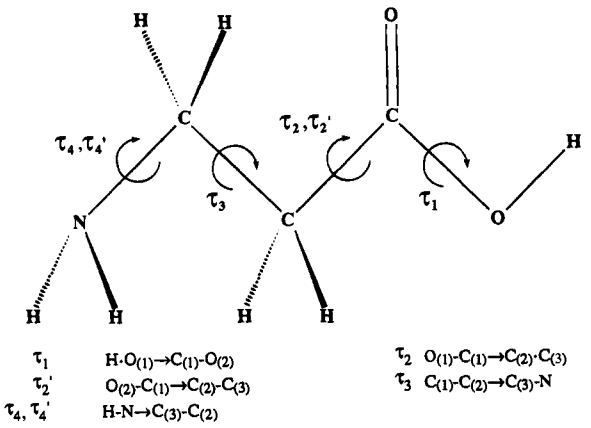

Figure 1. Parameters τ_1, τ_2, τ_3, and τ_4 defining the various conformations of β-alanine. All torsion angles are given clockwise with the eclipsed position as origin.

have not always yielded conformational predictions that are consistent with the experimental data available for these related species.[7,8]

Experimental Section

The Stark-modulated, free-expansion jet spectrometer is based on a design which has been described previously.[11] The mm-wave source was a phase-locked YIG-tuned FET microwave oscillator with frequency quadrupler. Stark modulation with electric fields up to 1600 V cm^{-1} was possible. Initial spectral searches were performed by broad-band scanning over the entire accessible frequency ranges of the spectrometer (48−55, 56−61, 62−66, and 70−72 GHz). Enhanced S/N ratios and precise line frequency measurements of detected absorption line profiles were obtained by signal averaging narrow-band repetitive scans collected using a microcomputer-based data acquisition system.

Crystalline β-alanine, obtained from Fluka, was vaporized at 230 °C and entrained in a stream of argon at a pressure typically of 30 kPa. The gaseous mixture was expanded supersonically through a 350 μm diameter nozzle held at 240 °C. Unlike alanine,[8] bulk solid samples would thermolyze during prolonged heating, and so it was necessary to feed the sample continuously into the vaporization chamber. The sample feed rate was typically 20 mg h^{-1}. Under these conditions no evidence of thermal decomposition was observed.

To form d_3-β-alanine, in which the labile hydroxy and amino protons have been exchanged, we dissolved β-alanine in 99.9 atom % deuterium D_2O and then removed the solvent under vacuum.

Ab Initio SCF Calculations

We have advanced the theoretical structure investigations of β-alanine with calculations performed at the HF/6-31G** level using the GAUSSIAN 90 package.[12]

The 20 symmetry-unique local minima that were found by Ramek et al.[10] using the 4-31G basis set provided initial molecular parameters for complete geometry optimizations in the 6-31G** based calculations. The structures were refined using threshold RMS force and displacement values of 1×10^{-8} Hartree pm^{-1} and 4×10^{-4} pm, respectively, as energy minima convergence criteria; conformational stability was confirmed by checking that the Hessian matrix possessed only positive eigenvalues. The shapes of the stable conformers found at the 6-31G** level are illustrated in Figure 2, and the calculated relative total energies, selected electronic properties and derived spectroscopic constants of each are listed in Table 1. It was found that the conformation corresponding to β-ala(XVII) was not a local minimum on the HF/6-31G** potential energy surface.

The extensive calculations in the literature on the previous member of the homologous series of *n*-alkylaminocarboxylic acids, glycine, reveal that particular conformers, e.g., glycine II, are unstable when basis sets employing polarization functions are used.[13] This is consistent with the view that calculations with such basis sets overestimate steric repulsions between eclipsed bonds.[14] The greater number of degrees of freedom of β-alanine can accommodate attractive intramolecular interactions without the concomitant formation of vicinal eclipsed bonds, thus avoiding conformers with sensitive basis set dependent geometries. However, we did find that β-ala(XVII) was not a local minimum on the HF/6-31G** potential energy surface, evidently the barrier to interconversion to β-ala(XIV) was no longer present.

The potential energy surface of β-alanine is partitioned by the conformation of the carboxyl group into two distinct regions: one with relatively low energy minima—β-ala-(I-XII)—in which the carboxyl group is *syn*-periplanar, i.e., $\tau_1 \approx 0°$, and the other consisting of high energy minima—β-ala-(XIII-XX)—with an *anti*-periplanar conformation, i.e., $\tau_1 \approx 180°$ (see Figure 1). However, the appreciable stabilization of β-ala-(V), $\tau_1 = 181.5°$, by internal hydrogen bonding makes this conformer an exception to the general result. The energy separation between the *trans* and *cis* conformations of formic acid has been experimentally determined[15] to be 16.38(36) kJ mol^{-1} and calculated to be 23.8 kJ mol^{-1} using the DZP basis set and the SCF method.[13] Thus while there is an appreciable energy expense associated with rotation of the hydroxy group, the HF/6-31G** results for glycine[7e] (which find that 13.6 kJ mol^{-1} separate glycine I and II; cf. experimental energy difference of 5.9 kJ mol^{-1}) suggest that the energy separation of approximately 30 kJ mol^{-1} between the two groups of β-alanine conformers is significantly overestimated.

Results and Discussion

It proved possible to assign every transition observed in the survey scans to spectra of two conformers of β-alanine, hereafter referred to as β-ala(x) and β-ala(y). The measured transitions are presented as supplementary data. The spectrum of β-ala-(x) was readily assigned by taking cognizance of the distinctive intensity patterns and frequency spacings of the dominant low-J, K_a (J = 4-9; K_a = 4) b-type and c-type transitions. The intensity of the strongest a-type transitions ($J = 11-17$; $K_a = 0-5$) of β-ala(y) were comparable to the most intense lines of β-ala(x). The b-type and c-type transitions of β-ala(y) were very weak.

The rotational constants derived from the two assigned spectra using the Watson S-reduced Hamiltonian are listed in Table 2. In the numerical least-squares fit of the spectroscopic constants the standard deviation in line frequencies was approximately estimated by adopting the convention that the standard deviation was equal to one tenth of the line width (typically 250 kHz), with proportionately larger uncertainties being given to under-modulated or poorly resolved lines. The small incidence of (observed − calculated) values which exceeded the estimated

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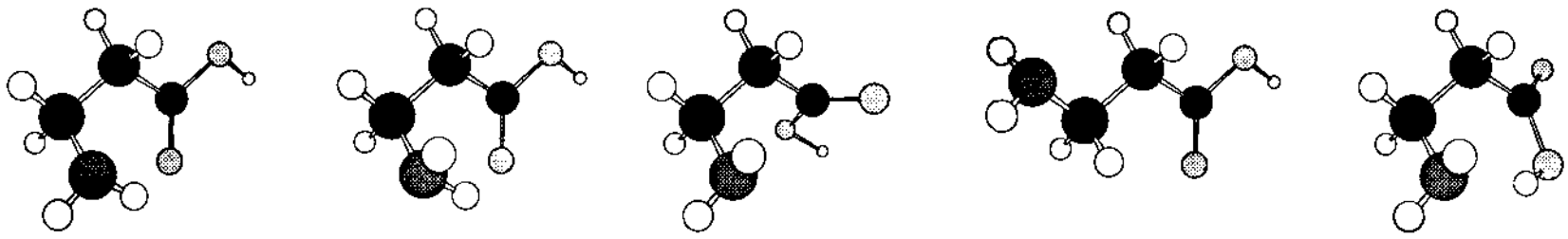
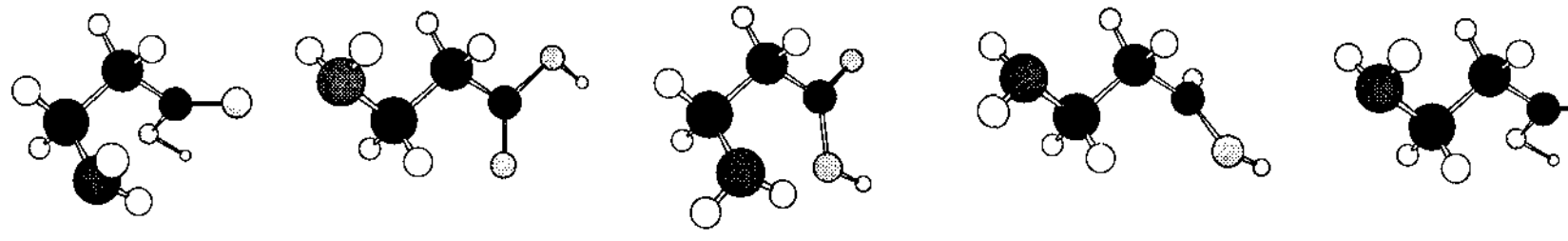


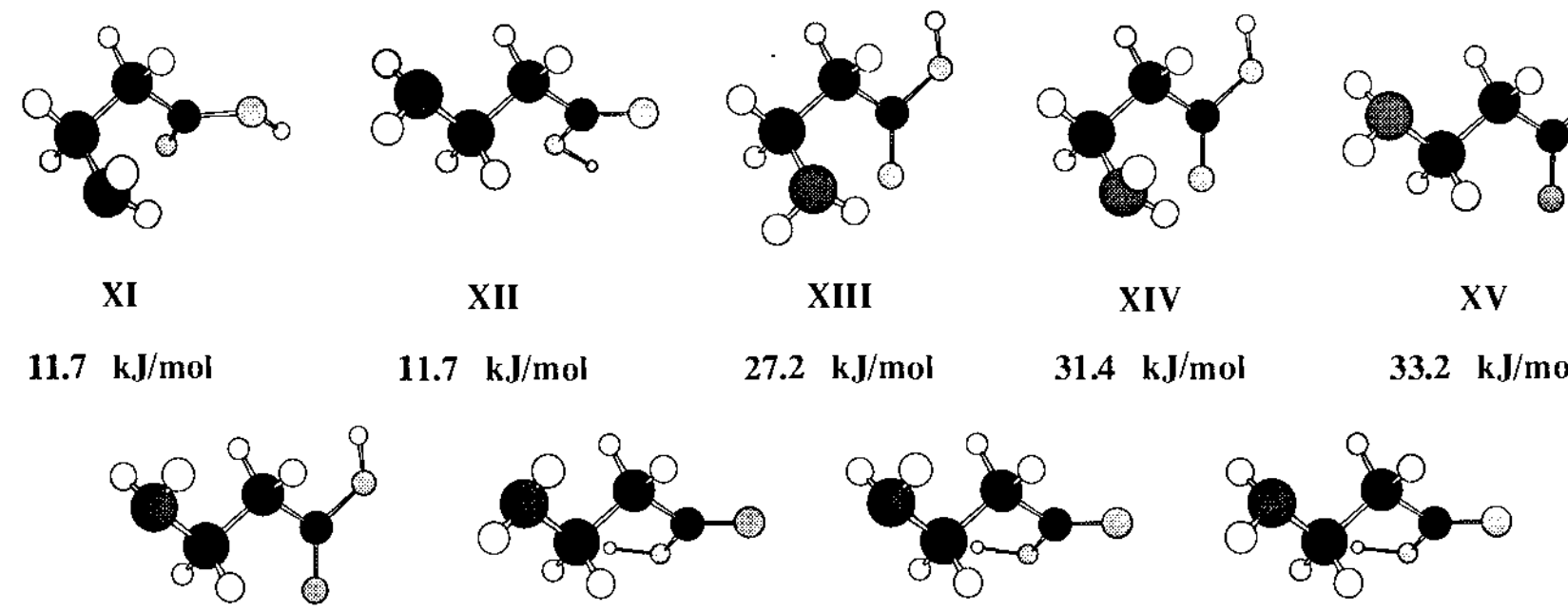
I **II** **III** **IV** **V**

0.0 kJ/mol **3.1 kJ/mol** **7.5 kJ/mol** **5.6 kJ/mol** **9.8 kJ/mol**



VI **VII** **VIII** **IX** **X**

9.8 kJ/mol **8.1 kJ/mol** **10.2 kJ/mol** **10.4 kJ/mol** **11.4 kJ/mol**



XI **XII** **XIII** **XIV** **XV**

11.7 kJ/mol **11.7 kJ/mol** **27.2 kJ/mol** **31.4 kJ/mol** **33.2 kJ/mol**

XVI **XVIII** **XIX** **XX**

37.4 kJ/mol **39.8 kJ/mol** **42.9 kJ/mol** **43.1 kJ/mol**

Figure 2. Nineteen conformers of β-alanine corresponding to local energy minima on the 6-31G** potential energy surface.

uncertainties indicated that the latter were actually greater than one standard deviation.

The results from the molecular orbital calculations reveal that a *trans* conformer ($\tau_3 = 180°$) would have rotational constants of $A \approx 8300-9600$ MHz, $B \approx 1800$-1900 MHz, and $C \approx 1500$-1700 MHz; whereas for *gauche* conformers ($\tau_3 = 60°$) $A \approx$ 5900-7500 MHz, $B \approx 2300-2500$ MHz, and $C \approx 1900-2300$ MHz. The *ab initio* rotational constants for alanine determined at the HF/6-31G** level were found to be within 3% of the experimental values. Since the smallest discrepancy between the rotational constants in Table 2 and those of the closest *trans* conformer, β-ala(XIV), is 13% we can almost definitively exclude a *trans* conformation for both β-ala(x) and β-ala(y).

Planar Moment of Inertia. It is possible to derive structural information regarding the mass distribution along the principal axes from the principal planar moments of inertia.[16] Since β-ala(x) and β-ala(y) are prolate near symmetric-tops (Ray's asymmetry parameter $\kappa = -0.868$ and -0.801, respectively), the greatest difference in mass distribution between the *gauche* and *trans* conformers occurs along the principal *a*-axis. The values of the planar moment of inertia of the principal *a*-axis, $P_a = {}^1/_2(-I_a + I_b + I_c)$, given in Table I clearly distinguish between *trans*, $P_a \approx 250-270$ uÅ^2, and *gauche*, $P_a \approx 170-205$ uÅ^2, conformations. The values of P_a for β-ala(x) and β-ala(y) are consistent with both being *gauche* conformers.

***d*$_3$-β-Alanine.** The spectra of the trideutero analogues of the two β-alanine conformers showed similar spectral characteristics to those of the parent species and were thus readily assigned; the derived spectroscopic constants and the associated changes in the rotational constants of the parent species are reported in Table 2. Since the changes in the rotational constants on deuteration are a function of the amino and hydroxy hydrogen positions, we can reduce the selection of possible gauche conformations using the results from the molecular orbital

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Table 1. *Ab Initio* HF/6−31G** Parameters[a] for β-Alanine

parameter[b]	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVIII	XIX	XX
τ_1	359.5	0.1	2.9	259.6	181.5	359.2	0.0	359.9	0.0	0.4	0.1	0.2	181.0	179.4	180.8	180.0	182.7	181.8	181.9
τ_2	181.1	180.8	55.9	176.8	320.8	54.9	180.0	23.7	303.4	66.2	291.6	64.4	184.1	181.3	180.2	180.0	77.2	74.2	78.9
τ_2'	1.5	1.0	233.9	356.5	142.3	235.0	0.0	201.9	123.7	247.0	110.8	244.6	4.8	1.4	0.2	0.0	259.2	256.1	260.9
τ_3	66.6	64.4	56.1	177.4	65.4	57.6	180.0	61.3	181.0	178.7	72.7	178.7	66.7	64.3	177.9	180.0	182.2	177.7	178.4
τ_4	287.4	57.8	188.4	189.4	193.2	53.7	60.4	282.5	189.1	61.0	62.1	190.2	287.0	58.3	192.1	60.8	292.1	58.9	191.8
τ_4'	168.7	296.8	67.9	70.5	73.2	292.0	299.6	163.6	69.8	300.1	302.4	71.0	168.3	296.9	73.3	−60.8	173.1	297.7	71.7
E_{rel}, kJ mol^{-1}	0.0	3.1	7.5	5.6	9.8	9.8	8.1	10.2	10.4	11.4	11.7	11.7	27.2	31.4	33.2	37.4	39.8	42.9	43.1
A, MHz	7490.3	7445.7	5990.2	9558.1	7320.8	5990.6	9507.4	6723.6	8833.1	8639.9	6726.1	8746.9	7352.2	7380.8	9327.4	9291.6	8369.0	8331.6	8359.2
B, MHz	2337.0	2306.3	2424.4	1863.4	2491.5	2373.2	1846.2	2400.1	1893.9	1877.5	2436.4	1880.5	2340.1	2310.0	1871.7	1854.8	1899.0	1884.9	1891.8
C, MHz	1985.6	1961.0	2310.8	1596.4	1987.0	2277.5	1588.2	2119.8	1666.9	1674.4	1990.4	1680.9	2002.6	1963.3	1596.7	1588.6	1696.6	1681.4	1696.8
P_a, uÅ^2	201.7	204.5	171.4	267.5	194.1	175.2	269.4	186.9	256.4	256.3	193.1	255.8	199.8	203.9	266.2	268.1	251.8	254.0	252.3
μ_a, D	0.1	1.7	2.5	0.5	6.6	0.1	0.6	1.9	1.4	0.5	0.5	1.4	1.2	0.5	2.6	1.5	4.0	3.2	4.0
μ_b, D	1.0	1.8	1.4	0.8	1.2	0.9	2.7	1.5	1.2	0.9	1.0	2.8	3.7	4.7	3.0	5.0	1.6	1.9	3.6
μ_c, D	0.8	0.9	0.9	1.1	0.6	0.7	0.0	1.9	1.0	1.4	1.5	0.0	0.4	1.2	0.9	0.0	0.3	2.5	0.6
μ_{tot}, D	1.2	2.6	3.0	1.4	6.8	1.1	2.8	3.1	2.1	1.7	1.9	3.1	3.9	4.8	4.1	5.2	4.3	4.5	5.4
χ_{aa}, MHz	1.831	−3.189	−2.144	2.163	−0.987	−3.125	−0.592	1.646	2.211	0.082	−1.712	2.229	1.794	−3.209	2.154	−0.578	2.249	0.180	2.253
χ_{bb}, MHz	0.819	1.656	1.935	−0.342	−0.774	1.174	−1.327	−0.044	1.807	1.025	2.176	−3.870	0.555	1.649	−0.905	−1.354	−0.172	1.354	−2.472
$\Delta(A)$, MHz[c]	−276.2	−411.1	−388.7	−271.7	−399.1	−436.6	−422.7	−473.4	−462.5	−478.6	−482.5	−431.6	−410.5	−539.5	−666.0	−803.3	−597.8	−638.4	−595.6
$\Delta(B)$, MHz[c]	−193.6	−168.7	−188.0	−181.4	−169.0	−139.9	−164.1	−152.7	−163.6	−150.5	−133.2	−168.2	−172.6	−147.8	−155.2	−137.0	−140.1	−123.6	−141.4
$\Delta(C)$, MHz[c]	−152.4	−120.7	−171.8	−136.4	−118.4	−121.9	−122.3	−147.6	−141.1	−130.2	−120.1	−138.4	−144.3	−112.3	−126.1	−112.5	−130.2	−117.6	−128.6
$\Delta(P_a)$, uÅ^2[c]	19.1	15.1	14.6	28.6	13.4	9.6	25.4	13.2	25.1	22.7	11.2	25.2	16.4	12.6	23.7	20.4	20.7	18.2	20.7

[a] Only the most important geometrical parameters defining the molecular conformation are given. [b] Bond angles and dihedral angles are in degrees. [c] Changes in the rotational constants and planar moment of inertia about the principal a-axis upon deuteration to d_3-β-alanine.

Table 2 Derived Spectroscopic Parameters[a] for the Main Species and Deuterated Analogues of β-Alanine

parameter	β-Ala(x)	β-Ala(y)	d_3-β-Ala(x)	d_3-β-Ala(y)
A, MHz	7267.8467(32)	7177.114(15)	7003.3332(24)	6804.652(13)
B, MHz	2335.1695(20)	2499.7265(36)	2145.5385(34)	2330.3189(46)
C, MHz	1986.7797(17)	1982.4560(18)	1835.4356(22)	1866.4603(24)
D_J, kHz	0.8388(36)	0.3855(43)	0.7304(89)	0.3335(57)
D_{JK}, kHz	−2.210(44)	0.121(79)	−2.652(22)	0.153(72)
D_K, kHz	19.98(11)	5.49(39)	21.234(72)	3.45(31)
d_1, kHz	*b*	−0.0861(36)	*b*	−0.0687(48)
d_2, kHz	−0.0420(25)	−0.0080(16)	−0.0267(65)	−0.0048(19)
rms of fit, kHz	38	78	24	39
no. of lines	31	44	31	39
P_a, uÅ^2	200.627	193.342	219.366	206.685
$\Delta(A)$, MHz	−264.51	−372.46		
$\Delta(B)$, MHz	−189.63	−169.41		
$\Delta(C)$, MHz	−151.34	−116.00		
$\Delta(P_a)$, uÅ^2	18.74	13.34		

[a] Numbers in parentheses represent one standard deviation as determined in the least-squares fit. [b] Statistically undetermined parameter (F-test, 99% confidence interval).

calculations (see Table 1). Only β-ala(I) reproduces changes in all three rotational constants which are in close agreement with those of β-ala(x). The concordance between the changes in P_a for these two conformers confirms this assignment. In the case of β-ala(y), the conformations of both β-ala(II) and β-ala(V) are found to give comparable changes in the rotational constants. However, the change in P_a for β-ala(y) favors the assignment to β-ala(V).

Hyperfine Splittings. The ^{14}N nuclear quadrupole hyperfine structure of the accessible transitions of β-alanine was sufficiently resolved only for certain weaker lines. Using the rotational constants of β-ala(x) and β-ala(y) with predicted *ab initio* quadrupole coupling constants (see Table 1) it was possible to calculate the expected hyperfine patterns associated with a particular electronic environment around the amino nitrogen atom. The multiplets for the *gauche* conformers β-ala(VIII), β-ala(XI), and β-ala(XIII) were similar to those for β-ala(I), while the only *gauche* conformer showing nuclear quadrupole splittings comparable to those for β-ala(V) was β-ala(VI). Thus, β-ala(II) can confidently be excluded as a possible conformation of β-ala(y). As shown in Figure 3, excellent agreement was obtained between the observed multiplets of β-ala(x) and β-ala(y) and those calculated using the theoretical coupling constants of β-ala(I) and β-ala(V), respectively.

Identification of Species. The degree of consistency between the spectroscopic constants, changes in the rotational constants on deuteration, and the ^{14}N hyperfine patterns conclusively show that β-ala(x) and β-ala(y) have the conformations of β-ala(I) and β-ala(V), respectively.

The observation of β-ala(x) in the supersonic jet is consistent with the *ab initio* molecular orbital calculations which uniformly predict it to have the lowest energy conformation. By contrast, the relatively intense a-type transitions of β-ala(y) enabled this particular conformer to be detected; the intensity of a rotational transition is proportional to the square of the relevant principal axis electric dipole moment component, and β-ala(V) is predicted to have an unusually large μ_a value of 6.6 D (see Table 1).

The experimental hyperfine patterns indicate that the observed conformers must be very similar in geometry to the assigned theoretical conformations. Based on the *ab initio* relative energies it was plausible that β-ala(x) might have had the vibrationally averaged conformation arising from the rotameric interconversion of β-ala(I) and β-ala(II). However, the ^{14}N hyperfine splitting is quite sensitive to changes in the principal axis components of the quadrupole coupling constants to the extent that the predictied hyperfine structure for the transition

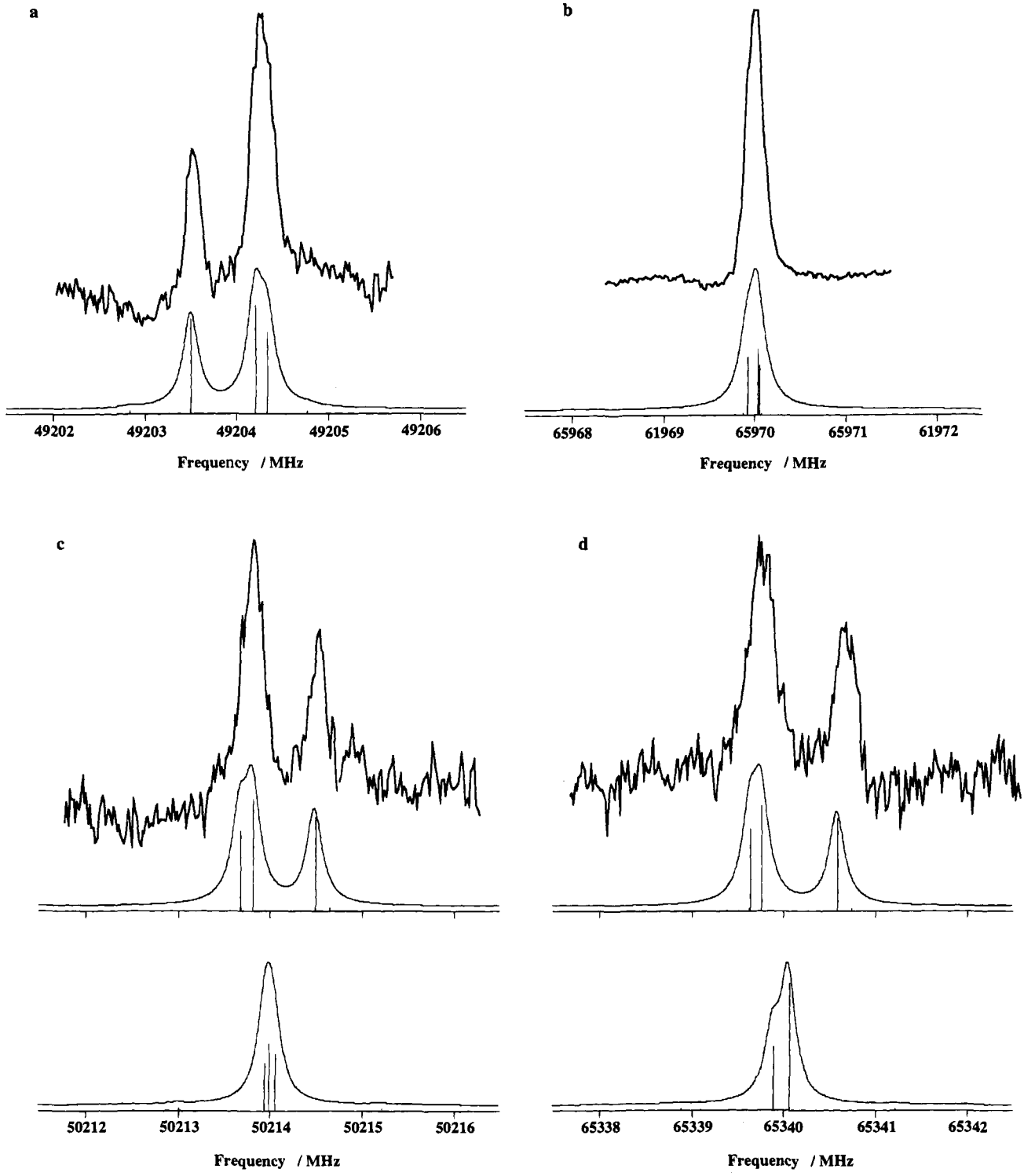

Figure 3. Observed and predicted ^{14}N nuclear hyperfine patterns of the $7_{2,6}$-$6_{1,6}$ (a) and $7_{4,3}$-$6_{3,3}$ (b) transitions of β-ala(x) and the $6_{2,4}$-$5_{0,5}$ (c) and $8_{2,6}$-$7_{0,7}$ (d) transitions of β-ala(y). The calculated patterns are based on the 6-31G** coupling constants (see Table 1) of β-ala(I) for (a) and (b), and β-ala(V) and β-ala(II), respectively, for the uppermost and lowermost profiles of (c) and (d).

in Figure 3a was unsplit and for the transition in Figure 3b was well resolved, when the quadrupole coupling constants of β-ala-(II) were used. Since the quadrupole coupling constants for the hypothetical vibrationally averaged structure would be different from those of both β-ala(I) and β-ala(II) the excellent agreement between the observed and predicted hyperfine multiplets shown in Figure 3(a,b) would not have been possible. Hence we conclude that the ground vibrational states of the two observed conformers of β-alanine do not involve any large-amplitude motions.

Within the detection limits of our spectrometer we saw no experimental evidence of any other gas-phase species. The curious absence of transitions arising from several conformers predicted to have energies comparable to the global minimum, particularly in view of the electric dipole moment advantage β-ala(II) has over β-ala(I), suggests that calculations of total energies at the Hartree−Fock level of theory are unreliable for the types of conformers considered here. The *ab initio* relative energy separation of two conformers of glycine,[13] glycine I and glycine II/III, is in good agreement with the experimentally

determined value[7] only when very elaborate basis sets are used at the post Hartree−Fock level, apparently the treatment of electron correlation effects is *sine qua non* if the calculations are to achieve quantitatively accurate energy determinations.

Conclusion

We have been able to establish that the observed rotational spectrum of β-alanine arises from two gaseous species which have *gauche* conformations. Results from *ab initio* molecular orbital calculations were instrumental in the successful elucidation of the geometry with respect to the amino and carboxyl functional groups. The two conformers involve the same types of intramolecular interactions found to occur in the observed gas-phase species of the α-amino acids glycine and alanine.

Molecular orbital calculations over the two basis sets that currently have been employed (4-31G and 6-31G**) could not reliably predict *a priori* which conformers should be observable on the basis of relative energies. It will be interesting to discover the effect of electron correlation energy on the relative stabilities of the β-alanine conformers.

No experimental evidence of a third conformer of β-alanine was found, as was also the case for both glycine and alanine. It is possible that the dynamics involved in forming a supersonic expansion have led to a reduction in the abundance of certain conformers, however, without details of the potential energy surfaces this issue cannot be satisfactorily resolved. The greater number of conformational possibilities of GABA suggests that more conformers should be amenable to experimental observations and thus offers the possibility of further refining our knowledge of the energy-ordering principles in amino−carboxyl interactions; we anticipate studying the shapes of the more complicated amino acids.

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Supplementary Material Available: Complete HF/6-31G** optimized geometries for each theoretical conformer and all the measured and assigned microwave transition frequencies used to derive the rotational constants reported in this work (10 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal and can be ordered from the ACS; see any current masthead page for ordering information.

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