

Alanyl Side Chain Folding in Phenylalanine: Conformational Assignments through Ultraviolet Rotational Band Contour Analysis

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Partially resolved ultraviolet rotational band contours associated with the $S_1 \leftarrow S_0$ origin bands of the six most populated conformers of jet-cooled phenylalanine have been recorded via resonant two-photon ionization. The strong dependence of their transition moment orientation on the conformation of the alanyl side chain has facilitated their structural assignment through simulations based upon *ab initio* computation. The S_1 lifetimes of all six conformers, measured through pump–probe delayed ionization, reveal an efficient nonradiative decay pathway in the most stable conformer, which is stabilized through a chain of intramolecular hydrogen bonds linking the side chain to the benzene ring.

1. Introduction

Ultraviolet “hole-burn” and mid-infrared “ion-dip” spectroscopy, coupled with *ab initio* computation, provides a powerful strategy for the structural investigation of flexible and particularly hydrogen-bonded biological molecules and their hydrated clusters, isolated in the cooled environment of a free-jet expansion.^{1–5} Very recently, its scope has been extended through the “fingerprint” region and toward the terahertz range through the use of a free electron laser source and exploited in a conformational reinvestigation of the amino acid tryptophan.⁶ Despite the power of infrared spectroscopy, however (interpreted through harmonic *ab initio* simulations), complete and unambiguous structural assignments of the full range of populated conformations cannot always be achieved in any given case and additional information must be sought.

One exciting approach developed by Dong and Miller⁷ is to generate oriented populations of molecular conformers (in helium nanodroplets) and determine their (molecular frame) vibrational transition moment alignments. A related, though more venerable approach uses high-resolution ultraviolet spectroscopy⁸ or partially resolved rotational band contour analysis⁹ to determine the rotational structure and hybrid character of individual conformers and thus their rotational constants and electronic transition moment alignments. The *ab initio* computation of both vibrational⁷ and electronic^{8,9} transition moment alignments is remarkably “robust”, converging rapidly with small basis sets to values in good agreement with experiment. In benzene derivatives especially, the alignment of the $S_1 \leftarrow S_0$, $\pi \rightarrow \pi^*$ electronic transition moment is particularly sensitive to the side chain structure and conformation¹⁰ not only through inertial axis rotation but, more importantly, through electronic perturbations which lead to ${}^1L_b/{}^1L_a$ state mixing¹¹ and strong

rotation of the electronic transition moments in the molecular frame. This is promoted both by “through-space” effects, arising from interactions between orbitals on the side chain and the π^* orbitals on the aromatic ring, and “through-bond” effects, generated by twisting of the side chain about the bond linking it to the ring.¹¹

Ab initio calculations (conducted at the CIS/6-31G*//HF/6-31G* level of theory) have identified the aromatic amino acid phenylalanine as a prime example of this behavior.¹² Its low-lying molecular conformations, first investigated by Martinez, Califano, and Levy¹³ using LIF saturation spectroscopy, were subsequently structurally assigned by Snoek et al.¹² using UV and IR ion-dip spectroscopy, coupled with *ab initio* computation. Five conformers, labeled A, B, C, D, and E by Martinez et al., were identified, together with a sixth, labeled X, which was not detected in the fluorescence emission but which appeared strongly in the resonantly enhanced two-photon ionization (R2PI) spectrum. The infrared-based structural assignments rested heavily on large shifts in the O–H vibration frequency associated with intramolecular hydrogen bonding, and very small conformationally dependent shifts in the antisymmetric N–H₂ stretch mode. Later investigations by S. K. Kim’s group^{14–16} characterizing its conformation-dependent ionization and hydration led to a revised structural assignment for the (weakly populated) conformer E which, unlike conformers X and B, did not exhibit a hydrogen-bonded, COOH \rightarrow NH₂ structure.

The present work provides a new and independent set of structural assignments based this time upon comparisons between the partially resolved rotational band contours of the R2PI spectrum of phenylalanine (including each of the six identified conformer origin bands and a few of its vibronic bands) and simulations based upon optimized *ab initio* calculations conducted at the HF/6-31G*//CIS/6-31G* level of theory. The S_1 lifetimes of each conformer have also been determined through pump–probe delayed ionization measurements at each of the origin bands, to rationalize the observed differences between the origin-band intensities of individual conformers displayed in their LIF and R2PI spectra.

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2. Methodology

2.1. Experiment. Phenylalanine samples were heated to 200 °C and co-expanded with argon (stagnation pressure, 60 Torr) through a pulsed nozzle (diameter, 0.8 mm) to form a pulsed supersonic jet which was collimated before entering the ionization chamber. Two-color, mass-resolved R2PI spectra were recorded using two Nd:YAG pumped dye lasers. The pulse energy of the frequency doubled, etalon-narrowed excitation laser (Lambda Physik Scanmate 2E, fundamental line width $\sim 0.02\text{ cm}^{-1}$) was limited to $150\text{ }\mu\text{J/pulse}$ to minimize saturation effects; that of the ionization laser (wavelength 270 nm) was 1–2 mJ/pulse but no ion signal was generated by the ionization laser only. Wavelength calibrations for rotational band contour scans were conducted by simultaneously recording the LIF spectrum of I_2 .¹⁷

Excited state (S_1) lifetimes were obtained from measurements of pump–probe delayed ionization and were determined by fitting the decay signal to a single-exponential function; any contribution arising from one-color two-photon ionization signals was avoided by restricting the fit to delay times $\geq 35\text{ ns}$.

2.2. Computation. The ab initio CIS calculations were conducted using Gaussian 98,¹⁸ and the data generated (rotational constants, transition moment alignments, hybrid composition) were employed to compute simulated rotational band contours for an asymmetric rigid rotor. The simulations assumed a rotational temperature of 6 K and spectral line widths of $\sim 0.05\text{ cm}^{-1}$.

3. Results

3.1. Computation. The calculated structures of the nine lowest energy conformers of phenylalanine, optimized at the MP2/6-311+G** level, together with the calculated alignments of the electronic transition moment, are reproduced in Figure 1. In each conformation of the alanyl side chain either the NH_2 group or the COOH group, or both groups, necessarily adopts a gauche conformation with respect to the aromatic ring and three subsets can be identified with $(\text{NH}_2, \text{COOH}) = (\text{gauche}, \text{gauche}), (\text{gauche}, \text{anti}),$ or $(\text{anti}, \text{gauche})$. The experimental rotational band contours will be sensitive both to the transition moment alignment with respect to the principal inertial axes a , b , and c (determining the hybrid character of the band system) and to the rotational constants of each conformer (reflected, for example, in the $^{\Delta\text{Ka}}Q_{\text{Ka}}$ subband head spacings ($\approx 2A'' - (B'' + C'')$) associated with a near symmetric prolate top). The dependence of the ground state rotational constants A'' , B'' , and C'' on the (anti/gauche) side chain conformation of the NH_2 and COOH groups is summarized in Figure 2; their values confirm the assignment of phenylalanine as a near-prolate top, with $\kappa = -(0.85 - 0.97)$. Side chain folding of the heavier COOH group strongly reduces A'' by around 30%, and increases by around 25% the much smaller rotational constants B'' and C'' . Changes in the conformation of the lighter NH_2 group have relatively little effect on the rotational constants. The calculated values of $A'' - (1/2)(B'' + C'')$ are listed in Table 1 for each of the nine lowest energy conformers, together with their transition moment components, $\mu_a^2, \mu_b^2, \mu_c^2$ and relative energies, E_{rel} .

3.2. Rotational Band Contours. The mass-selected two-color R2PI spectrum of phenylalanine in the region of the $S_1 \leftarrow S_0$ origin bands of the six conformers, labeled A, B, C, D, X, and E, is shown in Figure 3; several excited vibronic bands, labeled F, G, H, and I, can also be identified. The partially resolved rotational band contours of the origin bands of conformers X, D, and C recorded experimentally are compared in Figure 4

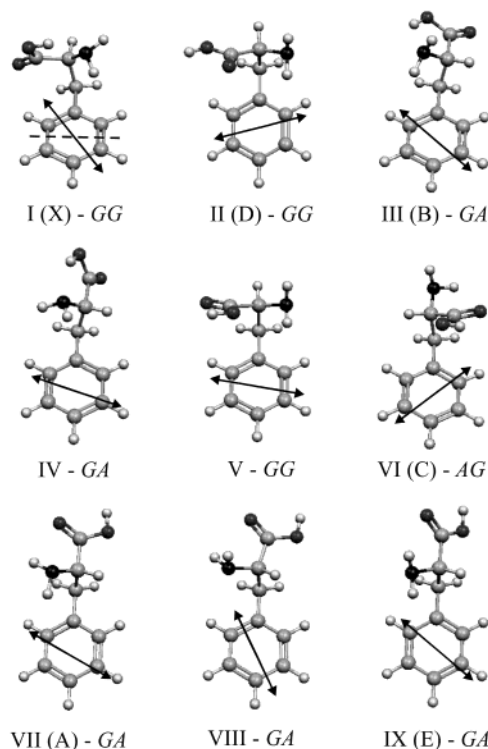


Figure 1. Computed structures of the nine lowest energy conformers of phenylalanine, calculated ab initio at the MP2/6-311+G** level of theory. The double-headed arrows indicate the alignment of the transition moment (calculated at the HF/6-31G**/CIS/6-31G* level of theory), projected onto the plane of the aromatic ring. The dotted line represents the b -axis in toluene.

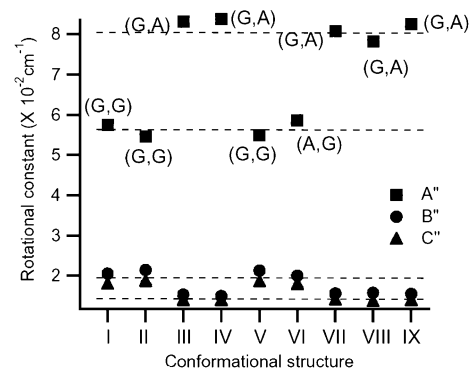


Figure 2. Dependence of ground-state rotational constants, A'' , B'' , and C'' , on the nine lowest energy conformational structures of phenylalanine. The side chain group conformations of $(\text{NH}_2, \text{COOH})$ are denoted by G = gauche and A = anti.

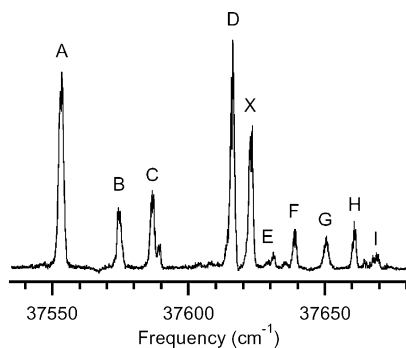
with those computed ab initio for the conformational structures I, II, and VI, respectively. Figure 5 shows the corresponding comparisons for the origin bands of conformers A, B, and E and the simulations based upon the ab initio computed structures, V or VI, II or IV, and VIII or IX.

The conformational structures of conformers X, D, and C were assigned previously, on the basis of infrared ion-dip spectroscopy coupled with high-level ab initio computation, to the structures I, II, and VI, respectively;¹² each structure accommodates a gauche COOH conformation. These assignments were consistent with the interpretation of subsequent experiments on the conformation-dependent hydration propensities and ionization potentials performed by Lee and co-workers.^{14–16} They are also fully consistent with the assignments based upon qualitative and quantitative comparisons between the experimental and computed rotational band contours dis-

TABLE 1: Optimized Relative Energies, E_{rel} , of the Nine Lowest Lying Conformers of Phenylalanine and Their Computed Band Contour Simulation Parameters

structure	$E_{\text{rel}}^a/\text{kJ mol}^{-1}$	$A'' - (1/2)(B'' + C'')^b/\text{cm}^{-1}$	$\mu_a^2:\mu_b^2:\mu_c^2^c$
I (G,G)	0.00	0.038 15	0.57:0.20:0.23
II (G,G)	0.63	0.034 54	0.00:0.98:0.02
III (G,A)	2.77	0.068 52	0.25:0.71:0.04
IV (G,A)	5.62	0.069 27	0.11:0.54:0.35
V (G,G)	4.57	0.034 88	0.03:0.90:0.07
VI (A,G)	3.11	0.039 55	0.32:0.33:0.35
VII (G,A)	6.59	0.065 84	0.14:0.80:0.06
VIII (G,A)	9.18	0.063 42	0.70:0.23:0.07
IX (G,A)	7.71	0.067 75	0.34:0.48:0.18

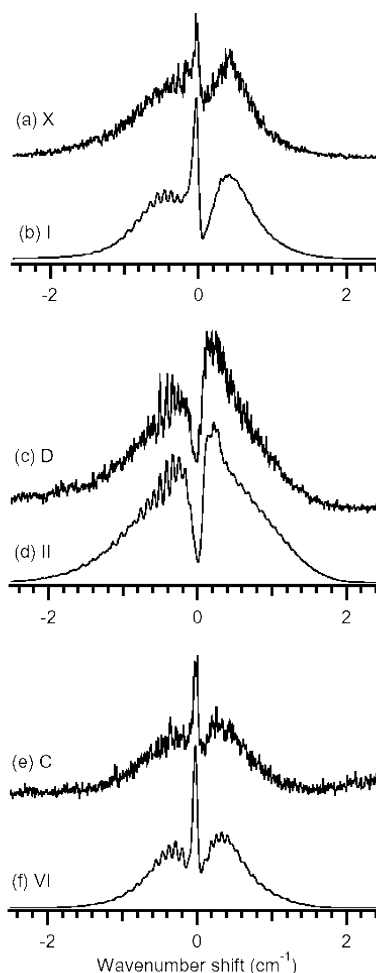
^a Optimized MP2/6-311+G** calculations, including zero point energy corrections. ^b HF/6-31G*. ^c CIS/6-31G**/HF/6-31G*.

**Figure 3.** Mass-selected R2PI spectrum of phenylalanine recorded in the region of the $S_1 \leftarrow S_0$ origin bands, labeled as A, B, C, D, X, and E. The bands F, G, H, and I are associated with transitions to vibronically excited levels.

played in Figure 4. In each case, it has been possible, at least partially, to resolve some of the $^{\text{P}}\text{Q}_{\text{Ka}}$ subband heads and determine their approximate spacings, which are all ca. 0.07 cm^{-1} ; these match the ab initio predictions listed in Table 1 and reflect the gauche conformation of the COOH group.

The experimental band contours for conformers A and B, shown in Figure 5, also reveal $^{\text{P}}\text{RQ}_{\text{Ka}}$ subband head progressions, now partially resolved in both the P and R branches. In contrast to the conformers X, D, and C, however, they are more widely separated with spacings ca. 0.13 cm^{-1} which closely match the values associated with an anti conformation of the COOH group (see Table 1). The close agreement between the rotational band contour of conformer B and that computed for the (gauche, anti) structure III supports the assignment proposed earlier by Snoek et al.,¹² but for conformer A, the earlier assignment to the (gauche, gauche) structure V needs to be revised. A comparison of its experimental band contour and the computed band contours for each of the four remaining (gauche, anti) structures, IV, VII, VIII, and IX, favors its assignment instead to structure VII. This is the only structure generating the high degree of type-b hybrid character displayed in Figure 5 (which also shows the computed band contour for the previously assigned structure V) together with the widely spaced subband progressions; the assignment to structure VII is also consistent with the absence of COOH \rightarrow NH₂ hydrogen bonding, revealed in its infrared spectrum.¹²

The final rotational band contour, associated with conformer E and shown in Figure 5g, displays a strongly mixed hybrid profile, indicating strong rotation of the $S_1 \leftarrow S_0$ transition moment away from the *b*-inertial axis. Its very low relative intensity in the R2PI spectrum precluded the recording of an infrared ion-dip spectrum, and the suggested, very tentative assignment to the hydrogen-bonded structure IV¹² was not consistent with the patterns of hydration and ionization behavior

**Figure 4.** Experimental partially resolved rotational band contours of (a) X, (c) D, and (e) C and simulated band contours associated with structures (b) I, (d) II, and (g) VI.**TABLE 2: Conformational Assignments and S_1 Lifetimes of Phenylalanine**

conformer	assignment	S_1 lifetime/ns
A	VII	88.8 ± 2.5
B	III	77.8 ± 3.3
C	VI	82.6 ± 2.5
D	II	77.3 ± 1.2
X	I	19.6 ± 2.3
E	IX	118 ± 13

subsequently revealed by Kim and co-workers.^{14–16} Although the rotational band structure is poorly resolved, the overall band profile is well and uniquely matched by the ab initio band contour associated with structure IX, shown in Figure 5h. The assignments are summarized in Table 2.

3.3. Lifetime Measurements. Table 2 lists the S_1 lifetimes determined for each of the six structurally assigned conformers. Those for conformers A–D are not significantly different from the corresponding lifetime (86.4 ns) of the chromophore molecule toluene,¹⁹ but that of conformer E ($118 \pm 13 \text{ ns}$) is noticeably longer and the lifetime of conformer X ($19.6 \pm 2.3 \text{ ns}$) is very much shorter. A comparison of the relative spectral intensities of the conformer origin bands in the LIF and R2PI spectra^{12,13} reveals particularly sharp differences for the bands X and E. In both the one-color¹² and two-color R2PI spectra (see Figure 2) band X is strong while band E is weak, but in the LIF spectrum band E is strong and band X cannot be discerned.¹³ The newly measured S_1 lifetimes indicate a low

TABLE 3: Computed “Through-Bond” and “Through-Space” Contributions to the Rotational Angle (degrees) of the $S_1 \leftarrow S_0$ Transition Moment, Relative to the Short Axis of the Aromatic Ring in Phenylalanine^a

conformer	carboxyl	amino	twist	$\Delta(\text{dihedral})$	Σ	Phe
I(X)	48	6	-15	-12	39	51
II(D)	12	-17	4	1	-1	-14
III(B)	13	5	24	15	42	41
IV	7	-4	19	13	22	20
V	31	-17	1	1	15	10
VI(C)	-26	3	-15	-12	-38	-37
VII(A)	17	-5	18	11	30	30
VIII	-4	42	-9	-3	29	65
IX(E)	8	20	5	5	33	41

^a “Carboxyl”, “amino”, “twist”, and “ Σ ” indicate the partial contributions and their sum; $\Delta(\text{dihedral})$ indicates the rotation (in degrees) of the computed ring-side chain dihedral angle away from orthogonality; “Phe” indicates the transition moment rotation angle computed for the complete molecule (see text). Positive values correspond to clockwise rotations.

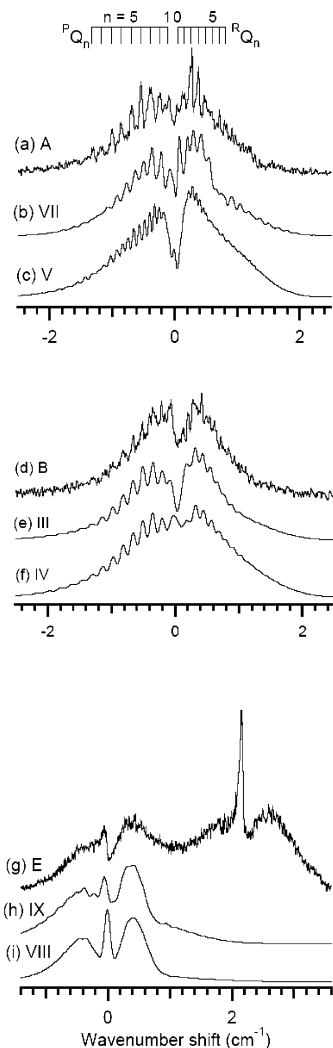


Figure 5. Experimental partially resolved rotational band contours of (a) A, (d) B, and (g) E and simulated band contours associated with structures (b, c) VII and V, (e, f) III and IV, and (h, i) IX and VIII. The partially resolved Q subband heads (denoted $\Delta K_a Q_{K_a'}$) are assigned by combination differences.

fluorescence quantum yield for the conformer X and a much larger quantum yield for conformer E.

4. Discussion

The electronic transition dipoles associated with the $S_1 \leftarrow S_0$ band origins in the anti conformers of 2-phenylethylamine and 3-phenyl-1-propionic acid are each aligned close to the short axis of the aromatic ring, reflecting the $^1B_2(^1L_b)$ character of

the S_1 electronic state of their parent chromophore, toluene. In contrast, the transition dipoles associated with the corresponding band origins of their gauche conformers are rotated away from the short axis, reflecting the influence of $^1L_a/^1L_b$ electronic state mixing.¹¹ The degree of rotation/mixing is markedly greater in the acid than in the amine, and in the latter case it is also modulated by the conformation of the amino group; the effect is greatest when the amino “lone pair” electron density is oriented toward the aromatic ring.⁹

Phenylalanine can be viewed as a composite of 2-phenylethylamine and 3-phenyl-1-propionic acid, and the interactions of its gauche oriented NH_2 or COOH groups with the aromatic ring might be thought, at some level of approximation, to be a simple summation of the two component contributions. In the [gauche, gauche] conformers II(D) or V, for example, where the two groups are opposed, there is only a small rotation of the transition moment away from the short axis of the ring (corresponding to the b -axis in toluene). Similarly, the rotational band contour of the [anti, gauche] conformer VI(C) qualitatively resembles that of the gauche conformer of 3-phenyl-1-propionic acid.¹⁰ On the other hand, in the [gauche, gauche] conformer I(X) where there is a cooperative chain of hydrogen bonds, $\text{COOH} \rightarrow \text{NH}_2 \rightarrow \text{aromatic } \pi\text{-electron ring}$, the transition moment rotation angle is very large.

To evaluate the contributions to the transition moment rotation away from the short axis of the ring, made either by “through-space” interactions associated with the two functional groups, or the “through-bond” interaction associated with twisting of the alanyl side chain away from orthogonality with respect to the aromatic ring, a partitioning scheme was devised similar to that employed in the earlier study of electronic state mixing.¹¹ Transition moment alignments and consequent rotation angles away from the short axis in the aromatic ring plane were calculated for “reduced” versions of phenylalanine, i.e., for 3-phenylpropionic acid, 2-phenylethylamine, and ethylbenzene, to quantify the contributions made by the COOH group, by the NH_2 group, and by twisting of the side chain, respectively: in each case their geometric configurations corresponded to the structures presented by each of the parent conformers. The results of the calculations are summarized in Table 3. With the exception of conformer VIII, where the lone pair on the amino group is directed toward the π -cloud, and where H-bonding from the amino to the carbonyl group may amplify the interaction in the “whole” molecule, the sum of the individual contributions (Σ in Table 3), compares quite well with the value calculated for the complete molecular conformer. In particular, there is a remarkably consistent correlation between the “twist” contribution to the transition moment rotation angle and the rotation angle of the alanyl side chain [$\Delta(\text{dihedral})$] away from an orthogonal orientation with respect to the aromatic ring. The

contributions made by the two functional groups in the [gauche, gauche] conformers II(D) and V are indeed seen to be opposed, and twisting of the side chain makes a negligible contribution. In the third [gauche, gauche] conformer, I(X), however, the carboxyl group exerts a large influence on the transition moment reorientation and is opposed *not* by the amino group but rather by the contribution introduced by twisting about the ring-side chain bond.

Further evidence of the “special” behavior of conformer I(X) is revealed in the short lifetime and low fluorescence efficiency of its S_1 electronic state—reflecting an effective nonradiative decay path. The corresponding hydrogen-bonded conformer of tryptophan, where there is also an enhanced $\text{NH}_2 \rightarrow \pi$ -electron interaction,²⁰ shows similar, though less dramatic behavior.²¹ The enhanced intersystem crossing to a triplet state promoted by the strong π -hydrogen bonding interaction in complexes of HCl and benzene or toluene²² encourages further pump–probe experiments designed to probe the (dark) triplet state.

The frequently poor correlation between the relative populations of jet-cooled molecular conformers and the ordering of their computed zero point relative energies has often been remarked (see, for example, the recent discussion of melatonin by Florio et al.²³) and attributed to the control exerted by collisional relaxation and the disposition of energy barriers on the conformational potential energy surface. The data shown in Table 1 provide another example of “missing conformers”. While the four lowest energy conformers I(X), II(D), III(B), and VI(C) lying in the relative energy range 0–3.1 kJ mol^{−1} are all populated, the other two conformers, VII(A) and IX(E), are calculated to lie considerably higher, at 6.6 and 7.7 kJ mol^{−1}, respectively; the absence of the intermediate conformations, IV and V (located at relative energies, 4.5 and 5.6 kJ mol^{−1}), suggests their facile relaxation, perhaps into the closely related conformations III(B) and II(D), respectively.

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