Rotational Spectra, Conformational Structure, and Methyl Internal Rotation Analysis of Biomolecules

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By

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DEDICATION

I dedicate this work to my late parents, beloved father Somasiri Marasinghe, beloved mother Priyangani Pathirana, my lovely wife Nirmala Gamage, and our precious sons, Theviru and Thevin for being with me in each single step in my life.

CHAPTER 1

INTRODUCTION

1.1 Rotational Spectroscopy

High-resolution rotational spectroscopy is an invaluable tool to probe the precise 3dimensional structures of molecules and molecular complexes in gas phase. It can be used to measure bond lengths, bond angles, and dihedral angles accurately because this technique is concerned with transitions between quantized rotational energy levels of the molecules that are low in energy with respect to the energies between electronic states and vibrational states of the molecules. Rotational transitions are directly related to the physical structure of the molecules because the rotational constants are inversely proportional to the moments of inertia of the molecule. The high resolution of the Fourier transform microwave (FTMW) spectrometer allows for the assignment of the rotational spectra arising from different molecules and molecular complexes, and their different conformers. Pure rotational spectroscopy is also known as microwave spectroscopy because the rotational transitions of most molecules fall within 3-300 GHz frequency range of the electromagnetic spectrum.

It is important to identify the structures of the molecules for many applications, especially in biochemistry and medical science, because the function of many pharmaceuticals depends on molecular interactions strongly influenced by molecular structure. For example, enzyme reactions are highly dependent on the structures and shapes of the molecules involved^{1,2}.

Rotational spectroscopy has been used to provide detailed structures for a larger number of small biomolecules. The spectra and structures of the two lowest energy conformers of amino acid glycine were found in early work^{3–8}. Two different conformers of valine were identified and analyzed

by microwave spectroscopy⁹. Microwave spectra were used to investigate methyl indole molecule and torsion-rotation spectra were assigned for a series of methyl indoles. Methyl torsional barriers for methyl substituted in different positions around the ring were determined from the tunneling splittings observed in the spectra¹⁰. Microwave spectroscopy is also crucial for detecting and identifying molecules in the interstellar medium. Detection of ammonia molecule in the interstellar medium by Cheung et al in late 1960s opened a new direction of using microwave spectroscopy to identify molecules and ions in the outer space¹¹. A recent study of rotational spectroscopy has been used to identify 2-methoxyethanol in the interstellar medium for the first time¹².

1.2 Motivation

Methyl internal rotation is one of most interesting features of small molecules with a methyl group, other than the structure determination. A large number of microwave spectroscopic studies in the last few decades have addressed this phenomenon. Methyl internal rotation was believed to be a free rotation along the rotor axis before Kemp and Pitzer found the potential energy barrier to internal rotation using thermodynamically calculated data for ethane in 1936¹³. They found a 1000 cm⁻¹ internal rotation barrier for ethane.

Many molecules with large amplitude motions have been investigated using Fourier transform microwave spectroscopy (FTMW) as it is capable of achieving the high resolutions needed to resolve small splittings arising from tunneling^{14–17}. The majority of previous studies were investigations of methyl internal rotation^{18–21}. Methyl internal rotation tunneling causes the rotational state to be split into symmetric (A) and antisymmetric (E) states, giving rise to separate rotational spectra, often separated by only 1 MHz or less. If the torsional barrier is larger, the A and E tunneling components will be relatively close to each other¹⁰, and smaller barriers will have the tunneling

components widely separated²². Thus the investigation of methyl internal torsion can provide information about the potential-energy barrier and the rotor-axis orientation, in addition to conformational and structural data.

The complexity of a molecular-rotational spectrum depends on several factors such as number and types of atoms, molecular symmetry, dipole moment and its projection onto the inertial axes, and methyl rotors²³. When an atomic nucleus has nuclear spin, I, of 1 or greater, nuclear spin angular momentum couples with rotational angular momentum, resulting in resolvable hyperfine structure composed of nuclear quadrupole hyperfine components. Assigning a rotational spectrum with methyl rotation splittings and nuclear quadrupole hyperfine structure is very challenging.

In the FTMW spectrometer, molecular samples are entrained and cooled in a supersonic expansion of argon (Temperature ~ 5 K). This environment provides less perturbed surroundings for molecular detection. Weakly bonded van de Waals complexes including molecular complexes with inert gases and hydrogen bonded molecular complexes with water can also be investigated in this environment.

1.3 Dissertation Outline

This dissertation describes four rotational spectroscopic studies on different biomolecules, molecular complexes, and other species. In these studies, we focus on methyl internal rotation, weak van der Waals interactions, and structural determination.

Chapter 2 provides the theoretical aspects of rotational spectroscopy/microwave spectroscopy. It discusses the theory behind electronic structure calculations, the Hamiltonian for molecular rotation, and the characterization of molecular structure from rotational spectra. Theoretical aspects of methyl internal rotation will be introduced in detail.

A detailed description of the mini-cavity Fourier transform microwave (FTMW) spectrometer used to record the spectra is given in Chapter 3. The four major components (pulse supersonic jet expansion system, the Fabry-Perot resonant cavity/vacuum chamber, the microwave circuit system, and custom-written computer program) and their importance of our custom-built FTMW spectrometer will be discussed here.

The rotational spectroscopic studies are described in Chapters 4 to 7. Chapter 4 is about conformational analysis of valine methyl ester (ValOMe). We discuss the conformational preferences of the molecule and calculate the methyl torsional barrier of two experimentally found conformers of this amino acid derivative. This project was a part of the analysis of structural information on amino acid methyl esters.

The mini-cavity FTMW spectrometer was used to record the spectrum of another amino acid derivative, proline methyl ester (PrOMe) as continuing the microwave study of amino acid methyl esters. Chapter 5 discusses, the experimental spectrum of PrOMe, the ring structure of the molecule, intramolecular hydrogen bonding, the methyl internal rotation, and the methyl barrier heights comparison between proline and prolinamide.

Chapter 6 describes another two interesting molecular systems: methyl heptanoate and methyl octanoate. Both molecules show two different conformational preferences which were identified experimentally. The methyl rotors splittings of these molecules were analyzed to find the V_3 barriers, 399.4(10) cm⁻¹ for C_s and 411(4) cm⁻¹ for C₁ conformer of methyl heptanoate and for the methyl octanoate, 399.2(13) cm⁻¹ and 409.3(26) cm⁻¹ for C_s and C₁ conformers, respectively. The detailed information of spectra and analysis of torsional barriers has been described in Chapter 6.

Rotational spectroscopy is capable of investigating not only the molecules but also the molecular complexes. In Chapter 7 we discuss rotational spectroscopic study of 2-methyl

aminoethanol water complex (2-MAE-H₂O). The rotational spectra, conformational preferences, and weak van der Waals interactions (Intermolecular hydrogen bonding) are being explained in detail.

Finally, Chapter 8 summarizes all projects and present the overall conclusions.

CHAPTER 2

THEORETICAL BACKGROUND

2.1 **Theoretical Aspects of Molecular Rotation.**

As our main goal of this research work is to analyze the structural information of amino acid methyl esters and other molecular complexes, we must have a better understanding of the relation between the rotational spectra, the molecular structure, and the theory behind the molecular rotation. The basic approach to describe the theory can be initiated with the classical expressions for the angular momenta and rotational energy. The derivation of the molecular structure relies on the classical moment of inertia as it describes the mass distribution of the molecules²⁴.

For a rigid molecular system, the classical angular momentum (**P**) is given by

$$\mathbf{P} = \mathbf{I}.\,\boldsymbol{\omega} \tag{2.1}$$

where I is the moment of inertia tensor and $\boldsymbol{\omega}$ is the angular velocity.

The moment of inertia tensor (I) is can be calculated with respect to a space-fixed x, y, and z Cartesian coordinate system.

$$\mathbf{I} = \begin{bmatrix} I_{xx} & I_{xy} & I_{xz} \\ I_{yx} & I_{yy} & I_{yz} \\ I_{zx} & I_{zy} & I_{zz} \end{bmatrix}$$
(2.2)

The individual components of the matrix tensor can be written as²⁵

$$I_{xx} = \sum m(y^2 + z^2)$$
 (2.3)

$$I_{yy} = \sum m(z^2 + x^2)$$
 (2.4)

$$I_{zz} = \sum m(x^2 + y^2)$$
 (2.5)

$$I_{xy} = I_{yx} = -\sum mxy \tag{2.6}$$

$$I_{zx} = I_{xz} = -\sum mxz \tag{2.7}$$

$$I_{yz} = I_{zy} = -\sum myz \tag{2.8}$$

where *m* is the mass of each atom of a molecule, and *x*, *y*, and *z* are its coordinates in the coordinate system with its origin at the center of mass. The total kinetic energy of the body is the summation of the kinetic energy of translational motion of the center of mass and the kinetic energy of the motion relative to the center of mass; these two motions can be separated. As the space-fixed axis system transforms to molecule-fixed axis system in molecular rotation, the principal moments of inertia can be presented as I_a , I_b , and I_c , where *a*, *b*, and *c* are the Cartesian axes of the molecular coordinate system. The minimum moment of inertia axis is about the *a*-axis and the largest moment of inertia maximum is about the *c*-axis. In the principal-axes coordinate system the inertia tensor becomes

$$\mathbf{I} = \begin{bmatrix} I_{xx} & 0 & 0\\ 0 & I_{yy} & 0\\ 0 & 0 & I_{zz} \end{bmatrix} = \begin{bmatrix} I_a & 0 & 0\\ 0 & I_b & 0\\ 0 & 0 & I_c \end{bmatrix}$$
(2.9)

Since the pattern of the rotational spectra depends on the principal moments of inertia, molecules can be categorized²⁶ according to the relationship between them as shown in the table 2.1.

Moments of Inertia	Molecule Type
$I_a = I_b = I_c$	Spherical Tops
$I_a = 0 < I_b = I_c$	Linear Tops
$I_a < I_b = I_c$	Prolate Symmetric Tops
$I_a = I_b < I_c$	Oblate Symmetric Tops
$I_a \neq I_b \neq I_c$	Asymmetric Tops

Table 2.1: Classification of molecules according to the moment of inertia of the system.

A molecule must possess a permanent dipole moment to observe rotational transitions in the microwave spectrum, so rotational spectra are not measurable for spherical tops.

The classical angular momentum is given by equation 2.1. When considering the moleculefixed principal axis system, a, b, and c, the individual components of the angular momentum are written as

$$P_a = I_a \omega_a \tag{2.10}$$

$$P_b = I_b \omega_b \tag{2.11}$$

$$P_c = I_c \omega_c \tag{2.12}$$

Therefore the total angular momentum can be written as the sum of angular momenta of each component:

$$P^2 = P_a^2 + P_b^2 + P_c^2 (2.13)$$

The rotational kinetic energy for a rigid rotor is given by.

$$E_r = \frac{1}{2} I \omega_{\cdot}^2 \tag{2.14}$$

It can be written in terms of the components of the principal axis system.

$$E_r = \frac{1}{2} \left(\frac{P_a^2}{I_a} + \frac{P_b^2}{I_b} + \frac{P_c^2}{I_c} \right)$$
(2.15)

In rotational spectroscopy, the molecular transitions are taken place between rotational energy levels, or the rotational eigenstates of the molecule. These eigenstates are given by eigenvalues of the Schrödinger equation²⁷ given by,

$$\widehat{H}_{R}(\Psi) = E_{I}(\Psi) \tag{2.16}$$

where E_I is the eigenvalue and Ψ is the wave function.

The Hamiltonian for a rigid body in the principal axis system can be given written as

$$\widehat{H}_{R} = \frac{1}{2} \left(\frac{\widehat{P}_{a}^{2}}{I_{a}} + \frac{\widehat{P}_{b}^{2}}{I_{b}} + \frac{\widehat{P}_{c}^{2}}{I_{c}} \right)$$
(2.17)

or using principal rotational constants, A, B, and C

$$\hat{H}_R = A\hat{P}_a^2 + B\hat{P}_b^2 + C\hat{P}_c^2 \tag{2.18}$$

The rotational constants are defined as²⁴

$$A = \frac{\hbar}{4\pi I_a}, \quad B = \frac{\hbar}{4\pi I_b}, \quad C = \frac{\hbar}{4\pi I_c}$$
(2.19)

where \hbar is the reduced Planck's constant. Principal moments of inertia are inversely proportional to the rotational constants. Hence, the rotational constants are directly related to the molecular structure.

2.1. (a) The rigid-rotor model for linear tops.

A linear molecule has one moment of inertia equal to zero and other two moments of inertia equal to each other ($I_a = 0$ and, $I_b = I_c$). Therefore the rotational Hamiltonian simplifies to

$$\widehat{H}_{R} = \frac{1}{2} \left(\frac{\widehat{P}_{a}^{2}}{I_{a}} + \frac{\widehat{P}_{b}^{2}}{I_{b}} + \frac{\widehat{P}_{c}^{2}}{I_{c}} \right) = \frac{\widehat{P}^{2}}{2I_{b}}$$
(2.20)

The eigenvalues of the system are given by²⁴

$$E_J = \frac{h^2}{8\pi^2 I_b} J(J+1)$$
(2.21)

where J is the quantum number of rotational angular momentum and h is Planck's constant.

Using the definition of B (equation 2.19) we can rewrite the eigenvalue equation as

$$E_I = hBJ(J+1) \tag{2.22}$$

The transition matrix $\langle \psi_{J''} | \mu | \psi_{J'} \rangle$ vanishes unless $J' = J'' \pm 1^{24,25}$ for a rotational transition from a lower rotational state to an upper rotational state of a linear top. Hence, the selection rule for rotational transitions of a linear top is,

$$\Delta J = \pm 1 \tag{2.23}$$

By taking the energy difference between the rotational energy states, the transition frequency can be obtained using the following equations.

$$\nu = \frac{E_{J'} - E_{J''}}{h} = B[J'(J'+1) - J''(J''+1)]$$
(2.24)

According to the selection rule, J' = J'' + 1. Therefore, ν can be simplified as follows.

$$\nu = 2B(J'' + 1) \tag{2.25}$$

Then the separation between two adjacent rotational transitions for a linear top is 2B.

2.1. (b) The rigid-rotor model for symmetric tops.

As mentioned earlier symmetric tops can be divided into two types according to the molecular geometry, prolate (e.g. NH₃, CH₃I, CHCl₃) and oblate (e.g.C₆H₆, C₄H₄).

For the prolate symmetric tops the moments of inertia relate as $I_a < I_b = I_c$. Hence the rotational Hamiltonian equation can be written as,

$$\widehat{H}_{R} = \frac{1}{2} \left(\frac{\widehat{P}_{a}^{2}}{I_{a}} + \frac{\widehat{P}_{b}^{2} + \widehat{P}_{c}^{2}}{I_{b}} \right)$$
(2.26)

As $P^2 = P_a^2 + P_b^2 + P_c^2$, the Hamiltonian can be rearranged as follows,

$$\widehat{H}_{R} = \frac{1}{2} \left(\frac{\widehat{P}_{a}^{2}}{I_{a}} + \frac{\widehat{P}^{2} - \widehat{P}_{a}^{2}}{I_{b}} \right) \quad or \quad \widehat{H}_{R} = \frac{\widehat{P}^{2}}{2I_{b}} + \frac{1}{2} \left(\frac{1}{I_{a}} - \frac{1}{I_{b}} \right) \widehat{P}_{a}^{2}$$
(2.27)

using that linear Hamiltonian $\hat{H}_R = \frac{\hat{P}^2}{2I_b}$, the Hamiltonian for a prolate top can be expressed as

$$\left[\widehat{H}_R\right]_{prolate} = \left[\widehat{H}_R\right]_{linear top} + \frac{1}{2} \left(\frac{1}{I_a} - \frac{1}{I_b}\right) \widehat{P}_a^2$$
(2.28)

But $\hat{P}_a = \hbar K$, where *K* is the quantum number for the projection of rotational angular momentum *J* onto the molecular symmetry axis *a*. Hence the rotational energy of the eigenstate of prolate symmetric top is given by²⁴

$$E_{JK} = hBJ(J+1) + \frac{\hbar^2}{2} \left(\frac{1}{I_a} - \frac{1}{I_b}\right) K^2.$$
(2.29)

The energy of an eigenstate can be rewritten using rotational constants (equation 2.19) as

$$\frac{E_{JK}}{h} = BJ(J+1) + (A-B)K^2$$
(2.30)

For oblate symmetric tops, the moments of inertia are related as $I_a = I_b < I_c$, and the rotational Hamiltonian can be written as,

$$\widehat{H}_{R} = \frac{1}{2} \left(\frac{\widehat{P}_{c}^{2}}{I_{c}} + \frac{\widehat{P}_{b}^{2} + \widehat{P}_{a}^{2}}{I_{a}} \right).$$
(2.31)

Rearranging the Hamiltonian for oblate tops²⁴ can be written as

$$\left[\hat{H}_R\right]_{oblate} = \left[\hat{H}_R\right]_{linear top} + \frac{1}{2} \left(\frac{1}{l_c} - \frac{1}{l_b}\right) \hat{P}_c^2, \qquad (2.32)$$

and the energy equation becomes

$$\frac{E_{JK}}{h} = BJ(J+1) + (C-B)K^2.$$
(2.33)

The selection rules for the rotational transitions of prolate and oblate tops are $\Delta J = 0, \pm 1$ and $\Delta K = 0$, because the transition marix $\langle \psi_{J''} | \mu | \psi_{J'} \rangle$ vanishes except under the conditions J' = J'' or $J' = J'' \pm 1$ and $K' = K''^{24}$.

2.1. (c) The rigid-rotor model for asymmetric tops.

When all three principal moments of inertia are nonzero and no two are equal, the molecule is an asymmetric top²⁴. In this situation, rotational frequencies can no longer be expressed as simple algebraic expressions. Instead, the asymmetric top is best understood by comparison to the prolate and oblate symmetric tops. The amount of asymmetry can be quantified by Ray's asymmetry parameter, κ , using the rotational constants (*A*, *B*, and *C*) as follows,

$$\kappa = \frac{2B - A - C}{A - C} \tag{2.34}$$

The limiting values for κ , -1 and +1, correspond to prolate and oblate symmetric tops respectively. The greatest asymmetry occurs when $\kappa = 0$. The degeneracy of the symmetric top energy levels with $\kappa > 0$ is lifted for asymmetric tops, and the states are labeled with the symmetric top κ values K_{-1} (prolate limit) and K_1 (oblate limit). The energy levels of symmetric tops are always (except $\kappa = 0$) degenerate, but they are separated in asymmetric tops. Therefore, an asymmetric top has (2J + 1) distinct rotational sublevels, while the symmetric top has only (J + 1) distinct rotational sublevels for each *J* value.

The Hamiltonian for the rigid asymmetric top is

$$\hat{H}_{R} = A\hat{P}_{a}^{2} + B\hat{P}_{b}^{2} + C\hat{P}_{c}^{2}$$
(2.35)

where *A*, *B*, and *C* the rotational constants, and \hat{P}_a^2 , \hat{P}_b^2 and \hat{P}_c^2 the angular momentum operators of rotation. The rearrangement of asymmetric top Hamiltonian results,

$$\hat{H}_{R} = \frac{1}{2}(A+C)\hat{P}^{2} + \frac{1}{2}(A-C)\hat{H}_{\kappa}$$
(2.36)

Where \hat{H}_{κ} is the reduced Hamiltonian, can be written as

$$\widehat{H}_{\kappa} = \widehat{P}_a^2 + \kappa \widehat{P}_b^2 - \widehat{P}_c^2 \tag{2.37}$$

2.2 Selection Rules.

The major selection rule is that the molecule must possess a permanent electric dipole moment for the electromagnetic radiation to cause a transition between rotational energy levels. The permanent electric dipole moment must have a non-zero value along the principal axes to observe each type of transition. These transitions are categorized as a-type transitions, b-type transitions, and c-type transitions. Selection rule for the dipole moment shown in table 2.2.

The allowed changes for the J quantum number are given by the specific selection rule

$$\Delta J = 0, \pm 1. \tag{2.38}$$

In addition to the selection rules for *J*, there are also restrictions on the changes that can occur for the K_{-1} and K_1 pseudo-quantum numbers due to the symmetric properties of the ellipsoid of inertia.

Table. 2.2. Selection rules for *a*-, *b*-, and *c*-type transitions.

Transition	Dipole Component	ΔK_{-1}	ΔK_1
a-type	$\mu_a \neq 0$	0, <u>±</u> 2,	<u>+</u> 1, <u>+</u> 3,
<i>b</i> -type	$\mu_b \neq 0$	±1, ±3,	±1, ±3,
<i>c</i> -type	$\mu_c \neq 0$	<u>+</u> 1, <u>+</u> 3,	0, ±2,

2.3 Centrifugal Distortion.

The initial treatment of the theory above regarded the molecular systems as nonvibrating rigid rotors. But in reality, molecules experience vibrational motion, even in the v = 0 level; these vibrations include both stretching and bending motions. Therefore the bond lengths and bond angles in molecules vary over time. Bond stretching increases the moments of inertia and decreases the rotational constants. Since the effect of centrifugal distortion is small compared to the rotational energy, centrifugal distortion is treated as a perturbation to the rigid rotor Hamiltonian, and the Hamiltonian can be represented as

$$\widehat{H} = \widehat{H}_R + \widehat{H}_d \tag{2.39}$$

where \hat{H}_R is the rigid rotor Hamiltonian and \hat{H}_d is the distortion energy Hamiltonian.

The resulting energies include centrifugal distortion via the distortion constant D_J , and the rotational energy for linear tops becomes

$$\frac{E_J}{h} = BJ(J+1) - D_J J^2 (J+1)^2, \qquad (2.40)$$

and the energy equations for prolate and oblate symmetric tops can be written as

$$\frac{E_J}{h} = BJ(J+1) + (A-B)K^2 - D_J J^2 (J+1)^2 - D_{JK} J (J+1)K^2 + D_K K^4$$
(2.41)

$$\frac{E_J}{h} = BJ(J+1) + (B-C)K^2 - D_J J^2 (J+1)^2 - D_{JK} J(J+1)K^2 + D_K K^4$$
(2.42)

where D_J , D_{JK} , and D_K are first-order centrifugal stretching distortion constants.

Watson's *A*-reduction Hamiltonian is used to predict and fit rotational spectra for most asymmetric molecules, and Watson's *S*-reduction Hamiltonian is more convenient for nearly symmetric molecules ($\kappa \approx 1$). In this work, Watson's *A*-reduction Hamiltonian is employed as all the species addressed here are more asymmetric^{28–30}. The quartic distortion constants are labeled as $\Delta_I, \Delta_{IK}, \Delta_K, \delta_I$, and, δ_K when using *A*-reduction Hamiltonian.

2.4 Methyl Internal Rotation.

Methyl internal rotation tunneling is one of the most interesting features that can be observed in rotational spectra. Methyl torsion is the rotation of a methyl group with respect to rest of the molecule along the bond connecting it. Quantum mechanical tunneling of states between equivalent potential energy minima result in a splitting into symmetric (labeled A) and antisymmetric (labeled E) tunneling states. Because of their different symmetries, these states give rise to separate rotational spectra, which often appears as a splitting of rotational transitions into two components (A and E). The tunneling splitting depends on the barrier height of the methyl torsion. Thus rotational spectroscopy can be employed to determine the barrier height calculation of methyl rotors.

The torsional angle (α) describes the internal rotation of two parts of a molecule relative to each other. As the two parts of the molecule rotate about the bond connecting them, the potential energy changes as a function of torsional angle (α). The number of equivalent configurations (N) in a complete rotation (2π) relative to each other depends on the symmetry of the molecule. The potential function for internal rotation can be expressed as a Fourier series expansion,

$$V(\alpha) = a_0 + \sum_{k=1}^{\infty} a_k \cos(kN\alpha), \qquad (2.43)$$

where a_0 and a_k are Fourier coefficients, a_0 defined as

$$a_0 = -\sum_{k=1}^{\infty} a_k \,. \tag{2.44}$$

The potential energy, V is 0 at $\alpha = 0, \pm \frac{2\pi}{N}, \pm \frac{4\pi}{N}$, and so on.

Thus, $V(\alpha)$ for *N*-fold rotor is

$$V(\alpha) = \frac{V_N}{2} (1 - \cos N\alpha) + \frac{V_{2N}}{2} (1 - \cos 2N\alpha) + \cdots$$
(2.45)

For rotors that have a threefold barrier $(N = 3) V(\alpha)$ can be rewritten

$$V(\alpha) = \frac{V_3}{2}(1 - \cos 3\alpha) + \frac{V_6}{2}(1 - \cos 6\alpha) + \cdots$$
(2.46)

Usually, only the first term of the cosine expansion is considered, as the V_6 term is much smaller than the V_3 term, and the potential function becomes

$$V(\alpha) = \frac{V_3}{2} (1 - \cos 3\alpha). \tag{2.47}$$

This form of the potential function for a threefold barrier (N = 3) has a sinusoidal shape as shown in Figure 2.1.



Figure 2.1. Schematic representation of the potential function and torsional energy levels of a 3-fold barrier.

2.5 **Torsional Energy Levels.**

There are three equivalent configurations of methyl internal rotation due to the three-fold symmetry of the methyl group. If these configurations are independent each other, the torsional energy levels would be triply degenerate as shown in the Figure 2.1. The wave equation for the internal rotation satisfies the one-dimensional Schrödinger equation.

$$-F\frac{d^2 U(\alpha)}{d\alpha^2} + \left[\frac{V_3}{2}(1 - \cos 3\alpha) - E\right]U(\alpha) = 0$$
(2.48)

Where α is the torsional angle, $F = \frac{\hbar^2}{2I_r}$, I_r is the reduced moment of inertia, V_3 is the height of the three-fold barrier, and $U(\alpha)$ is the wave function.

For the very small barrier case of $V_3 \approx 0$, (free rotation), the equation reduces,

$$\frac{d^2 U(\alpha)}{d\alpha^2} + \left(\frac{1}{F}\right) E U(\alpha) = 0, \qquad (2.49)$$

And the solution is written using a normalization factor, A.

$$U(\alpha) = Ae^{imx} = A(\cos m\alpha + i\sin m\alpha).$$
(2.50)

Then the energy is given by,

$$E = Fm^2 \tag{2.51}$$

For the function $U(\alpha)$ to be well behaved, it must satisfy the boundary condition

$$U(\alpha) = U(\alpha + 2\pi) \tag{2.52}$$

and *m* is required to take on the values $m = 0, \pm 1, \pm 2, \pm 3, ...$

The normalization factor A can be evaluated given the normalization condition,

$$A = \frac{1}{\sqrt{2}}\pi\tag{2.53}$$

For the large barrier case of $V_3 \rightarrow \infty$ (harmonic oscillator), the cosine function can be expressed as a Taylor series expansion for smaller values of α as,

$$\cos 3\alpha = 1 - \left(\frac{9}{2}\right)\alpha^2 + \left(\frac{27}{8}\right)\alpha^4 + \cdots$$
 (2.54)

Then the potential function for a barrier with three-fold symmetry may be written as,

$$V(\alpha) = {9 \choose 4} V_3 \alpha^2 - {27 \choose 16} V_3 \alpha^4 + \dots$$
(2.55)

The wave equation reduces to the given form in the following equation as only the first term of the expansion retains in this harmonic approximation.

$$\frac{d^2 U(\alpha)}{d\alpha^2} + \frac{1}{F} \left[E - \frac{1}{2} \left(\frac{9}{2} V_3 \right) \alpha^2 \right] U(\alpha) = 0$$
(2.56)

This equation has the wave form of simple harmonic oscillator. Then the energy can be written as,

$$E = 3(V_3F)^{\frac{1}{2}} \left(v + \frac{1}{2} \right)$$
(2.57)

where v is the harmonic oscillator quantum number, which allows, 0, 1, 2, 3, ...

The frequency, ν of the torsional oscillation can be taken as follows as the energy levels are approximately act as harmonic oscillators.

$$\nu = \frac{3}{2\pi} \left(\frac{V_3}{2l_r}\right)^{1/2}$$
(2.58)

Since the internal rotation become torsional oscillation for an infinite barrier, each torsional state is triply degenerate. For finite torsional barriers with $E < V_3$, the wave functions have some probability of tunneling through the barriers and partially lifting the degeneracy. The triply degenerate torsional levels are spit into two levels, a non-degenerate *A* state and a doubly degenerate *E* state. The correlation between free rotor ($V_3 \approx 0$) case and the harmonic oscillator ($V_3 \rightarrow \infty$) case is illustrated in Figure 2.2.



Figure 2.2. Correlation between the energy levels of free internal rotation and harmonic torsional oscillation.

In free rotor case $(V \rightarrow 0)$, the states are doubly degenerate except for the state m = 0.

2.6 Nuclear Quadrupole Hyperfine Structure.

The nuclear charge distributions of isotopes with nuclear spin I = 0 or $\frac{1}{2}$ (e.g. ¹H, ¹²C, ¹⁶O, ¹⁵N, etc.) are spherical. If either the electronic charge or the nuclear charge is spherical about the nucleus, no nuclear quadrupole hyperfine structure can be seen. Molecules containing isotopes with nuclear spin of 1 or greater (e.g. ²H, ¹⁴N, ³⁵Cl, etc.) show the nuclear quadrupole coupling effect which results from the interaction between a nonspherical distribution of nuclear charge and the electric field gradient at the nucleus. Nuclear quadrupole hyperfine structure may be used to obtain
information about electronic structure and the chemical bonds because it provides a measure of the molecular field gradient. In the gas phase, nuclear quadrupole interactions are different for each rotational state, and each rotational transition splits into hyperfine components that can be assigned and used to determine principal components of the quadrupole coupling constant tensor (χ).

The total angular momentum (F) results from the coupling of rotational angular momentum (J) and nuclear spin angular momentum (I). These vectors are quantized, and the total angular momentum quantum number (F) is restricted to certain values

$$\boldsymbol{F} = \boldsymbol{I} + \boldsymbol{J} \tag{2.59}$$

$$F = J + 1, J + I - 1, J + I - 2, \dots, |J - I|.$$
(2.60)

The Hamiltonian (H) can be written as

$$H = H^A + H_0 \tag{2.61}$$

where H^A is the Watson A-reduction Hamiltonian and H_Q accounts for the quadrupole interaction.

The Hamiltonian describing the nuclear quadrupole interaction (H_Q) is given by

$$H_Q = \frac{eQq_J}{2J(2J-1)I(2I-1)} \left[3(I.J)^2 + \frac{3}{2}I.J - I^2 J^2 \right],$$
(2.62)

where

$$I.J = \frac{1}{2}(F^2 - J^2 - I^2)$$
(2.63)

and

$$I^{2}J^{2} = J(J+1)I(I+1)$$
(2.64)

where Q is the nuclear quadrupole moment, e is the charge of an electron, and q_J is the electric field gradient at the nucleus.

The energy related to the H_Q is given by

$$E_Q = \frac{eQq_J}{2J(2J-1)I(2I-1)} \left[\frac{4}{3} C(C+1) - J(J+1)I(I+1) \right]$$
(2.65)

where

$$C = F(F+1) - J(J+1) - I(I+1)$$
(2.66)

The quadrupole coupling constant tensor (χ) is represented in equation 2.67, and it can be determined by rotational spectroscopy.

$$\boldsymbol{\chi} = e Q q_J \tag{2.67}$$

In the principal axes system, the diagonal elements of the quadrupole coupling tensor are $\chi_{aa} = eQq_{aa}, \chi_{bb} = eQq_{bb}$, and, $\chi_{cc} = eQq_{cc}$

The relationship of these diagonal elements of the χ is given by the 2.68.

$$\chi_{aa} + \chi_{bb} + \chi_{cc} = 0 \tag{2.68}$$

CHAPTER 3

EXPERIMENTAL INSTRUMENTATION

3.1 **Cavity-based Fourier Transform Microwave Spectrometer.**

The rotational spectra of the molecules and the molecular complexes presented in this dissertation were recorded with a custom-built mini cavity-based Fourier transform microwave (CB-FTMW) spectrometer (shown in Figure 3.1). The CB-FTMW spectrometer in the Tubergen lab at Kent State University was built in 2004^{31} , based on the original design by Ball and Flygare³² and modeled after the mini-instrument setup at NIST³³. This CB-FTMW spectrometer is capable of recording the microwave spectra of molecules in the 9 - 23 GHz frequency range. The spectrometer consists of four major components, (1) a Fabry- Pérot resonant cavity in a vacuum chamber, (2) a pulsed supersonic jet-expansion system, (3) microwave circuitry, and (4) custom-written computer software.

3.1. (a) The Fabry-Pérot resonant cavity.

The Fabry-Pérot resonant cavity consists of two concave mirrors (7.5 in. diameter, and 12.0 in. spherical radius of curvature) separated by 30 cm inside the vacuum chamber. These aluminum mirrors were polished using diamond-tip machining. It is crucial for resonant cavity mirrors because highly polished mirrors with high Q factor (a measure of radiation loss in the cavity^{32,34}) minimizes the diffraction loss of microwave radiation.





Figure 3.1. (A) The cavity-based Fourier transform microwave spectrometer at the Tubergen Lab at Kent State University, including instrument rack and the computer system (B) A closer image of the vacuum chamber and jet expansion system.

One mirror is stationary while the other one is movable over a two-inch range to establish resonance at a desired frequency. The movable mirror is mounted on two steel rails using ball bearing jackets and moved using an Oriel motorized micrometer powered by NI PCI-6601 counter timer board. The stationary mirror is fixed next to the sample nozzle inside the vacuum chamber. The radiation enters the resonant cavity reflects back and forth between the mirrors, increasing the effective path length for molecular interactions.

The two mirror system resides inside a six-way cross vacuum chamber which is formed by a 15.5 in. long, 8 in. diameter tube with four 6 in. diameter ports. The motion platform for the movable mirror is resides inside the vacuum chamber. The vacuum inside the chamber (pressure < 0.001 torr) is created using Varian VHS-6 diffusion pump (2400 Ls⁻¹) and a two-stage Edwards E2M30 rotary pump. The diffusion pump creates a vacuum gradient from top to bottom to trap gaseous molecules inside the chamber and move to the bottom when they collide with the water-cooled wall of the diffusion pump. Gases are removed from the system with help of the rotary pump connected at the bottom of the diffusion pump.

3.1. (b) The pulsed supersonic jet-expansion system.

The CB-FTMW spectrometer uses inert carrier gases like argon (Ar), helium (He), or helium/Neon mixture (He/Ne) to introduce the sample into the resonant cavity in the vacuum chamber via supersonic jet-expansion³⁵. This system consists of a reservoir nozzle which is a modified Series-9 General Valve, mounted outside of the vacuum chamber through the stationary-mirror flange to the inside (shown in Figure 3.2). The expansion (gas) enters into the resonant cavity through a 0.182 in. diameter hole in the stationary mirror. The sample nozzle can be heated up to 200 °C using a Watlow STB1A1A3-A12 band

heater and the temperature is controlled by an Omega CN8201 temperature controller. This

heating



Figure 3.2. Top panel shows the schematic of the Fabry-Pérot resonant cavity in the vacuum chamber and an illustration of the supersonic jet-expansion (A-gas inlet, B-sample nozzle, C-stationary mirror, D-movable mirror, E- supersonic jet-expansion, and F-antennas). Bottom panel shows the actual side view of the vacuum chamber of the spectrometer.

system increases the vapor pressure of a sample and reduces the amount of signal averaging needed to obtain a spectrum with good signal to noise.

The supersonic jet expansion allows the sample molecules with random thermal energies in the sample nozzle to become a direct mass flow inside in the vacuum chamber (shown in Figure 3.2)³⁵. Supersonic jet-expansion isolates the gas molecules and cool molecular rotational and vibrational degrees of freedom inside the vacuum chamber with little or no condensation³⁶. The supersonic expansion directly cools only the translational motion of gas molecules. Internal degrees of freedom are cooled through molecular collisions present at the beginning of the expansion, then the molecular rotations are cooled faster than the molecular vibrations. This happens because of the much faster equilibrium between translational motion and the molecular rotations. Inside the vacuum chamber the temperature is about 5 Kelvin, which is far below than molecular freezing points.

3.1. (c) Microwave Circuitry.

The microwave circuit is used to irradiate the molecular expansion and detect the experimental signals. The schematic diagram of the irradiation and detection microwave circuitry is shown in Figure 3.3. An Agilent Technologies microwave synthesizer (Agilent E8247C PSG CW) is used to generate the microwave radiation (typically 15 dBm) and the radiation is directed into a pin diode switch S1 (Sierra Microwave Technologies SPDT-SFD0526-011). The microwave synthesizer generates two signals, a v_0 MW frequency and a 10 MHz reference signal. Using a frequency multiplier (Techtrol Cyclonetics FXA217-30), the 10 MHz frequency is upconverted to 30 MHz. The v_0 MW frequency is routed through the *a*-branch of the switch S1 and the 30 MHz frequency is routed through the diode switch S2

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(SPST Mini-Circuits ZYSW-2-50DR) into a single sideband mixer (SSBM- Miteq

SM0226LC1A). The output of SSBM (ν_0 +30 MHz) is amplified by Miteq low-noise A1 amplifier (AFSM3-02001800-40-8P-C) and enters the resonant cavity through the SPDT pin diode switch S₃ (SFD0526-011) that is located outside the chamber before an L-shaped antenna (~1.5 cm long center-conductor wire of an RF coaxial cable bent to L-shape) which directs microwave radiation into the cavity (irradiation pulses typically last 0.8 µs). The same L-shaped antenna is used for irradiation (ν_0 +30 MHz) and detection of the raw signal (ν_0 + 30 MHz + δ), where δ is the offset of the molecular signal from the irradiation frequency.

The signal detection route of the microwave circuitry is typically opened 400 µs after the irradiation pulse. The signal (v_0 + 30 MHz ± δ), is routed the *b*-branch of the S3 pin diode switch into the low-noise amplifier A2 (Miteq JS4-10002600-22-5A) located directly after switch S3. The amplified signal down converted with v_0 MW frequency in an image rejection mixer IR (Miteq IR0226LC1A). Therefore the output signal from the IR mixture is the offset of the molecular signal from the irradiation frequency (δ) superimposed on the 30 MHz sideband frequency (30 MHz ± δ). Then the output signal is filtered by a Minicircuits band-pass filter (SIF-30, amplified by a Miteq Au-1494 RF amplifier A3 (56dB gain) and routed into a National Instruments 100 MHz, 8-bit digitizing board (NI5112). 40000 channels are digitized at a rate of 100 MHz resulting in a digital frequency resolution of 2.5 kHz. The FID signal is recorded for 400 µs. The timing of the gas pulse, irradiation pulse, and detection are controlled by two Stanford Research Systems Digital Delay Generators (DG535) which are phase locked to the Agilent frequency generator through the 10 MHz reference signal.

3.1. (d) Spectrometer controlling software.

The FTMW spectrometer is interfaced to the computer using custom-written LabVIEW software, which controls the frequency and digital delay generators (through a National Instrument

GPIB board 778032-01). The computer program is also used to set the timing of the experiment and position the mirror. The software can perform time-domain signal averaging, frequency scanning by stepping the irradiation frequency and the resonance cavity, Fourier transformation, displays and saves the data. The cavity is adjusted to resonate with the irradiation frequency by changing the position of movable mirror while monitoring the voltage output of a Herotek Inc. Schottky detector (DHM265AA). The L-shaped antenna mounted at the center of the movable mirror detects the resonant signal.

The coaxial arrangement of the supersonic expansion and the cavity axis splits the signal into a doublet as shown in Figure 3.4. This is due to the Doppler effect taken place between the two mirrors. The Doppler doublets arises from molecules interacting with radiation coming from in front and from behind as the radiation resonates in the cavity. The Full width at half maximum (FWHM) of each component of the doublet is ~13 kHz. Below 10.5 GHz, the spectrometer signals are weak due to low Q factor of the cavity. The low-noise amplifier A1 falls off above 20 GHz reducing signal strength at high frequencies. Weak signals below 10.5 GHz or above 20 GHz may sometimes be found by increasing the signal averaging. The vacuum chamber is capable of maintaining the pressure below 0.001 torr with gas pulse repetition rates up to 15 s⁻¹, and the instrument can scan 1 GHz range in about 12.75 hours while averaging 100 shots per scan segment of 0.5 MHz.



Figure 3.3. The Schematic diagram of the Microwave Circuitry of the mini cavity FTMW spectrometer at the Tubergen Lab.



Figure 3.4. The $J = 1 \leftarrow 0$ rotational transition of ¹⁸OCS in natural abundance recorded with 100 averaged shots is showing the Doppler components.

3.2 **Other techniques.**

In addition FTMW spectroscopy, there are few other techniques were employed in the research work described in this dissertation. The solvent-solvent extraction method was used extract the methyl esters from their hydrochloride salts. Specific details of extraction processes for samples will be discussed in each chapter. NMR spectroscopy (Bruker 400 MHz NMR) was used to validate the samples before recording rotational spectra. ¹H-NMR and ¹³C-NMR spectra were obtained for all the extracted samples to confirm the extracted samples were pure and ready to use in recording rotational spectrum.

3.3 **Computational Methods.**

Rotational spectroscopy is always combined with computational chemistry because experimental rotational spectra are assigned with the help of the predicted spectra from *ab initio* calculations. These calculations begin with structural optimizations for different plausible starting structures of molecules and molecular complexes. The starting structures of all the molecules addressed in this dissertation were created using the molecular modeling software Avogadro. The ab initio input files were optimized by using different levels of theory and different basis sets (typically, ω B97XD/6- 311++G(d,p) and MP2/6- 311++G(d,p) levels of theory). *Ab initio* calculations were performed using the GAUSSIAN 16 program³⁷ running on the Owens cluster at the Ohio Supercomputer³⁸. The flow chart in Figure 3.5 helps to visualize the relation between computational and experimental paths in assigning rotational spectra.



Figure 3.5. The summarized flowchart of assigning a rotational spectrum of a molecule.

CHAPTER 4

IDENTIFICATION OF TWO STABLE SIDE-CHAIN ORIENTATION OF VALINE METHYL ESTER BY MICROWAVE SPECTROSCOPY

4.1 Introduction

High-resolution rotational spectroscopy provides precise structural and conformational information of biomimetic molecules in the gas phase. Microwave spectra were used to determine the structures of two different conformations of the amino acid glycine^{3–8}. The lowest energy conformation was found to have intramolecular hydrogen bonds from the amine to the carbonyl oxygen⁷, and a higher energy conformation – observed first because of its larger dipole moment – has an intramolecular hydrogen bond from the carboxylic acid to the amino nitrogen^{3,8}. Laserablation Fourier-transform microwave spectroscopy has since been used to record rotational spectra and determine precise conformational structures for many neutral amino acids³⁹ including valine⁹. The two conformers of valine that were identified from the microwave spectrum have the same orientation of the isopropyl side chain ($\chi_l=H^\beta-C^\beta-C^\alpha-H^\alpha\approx$ -60°) for each of the intramolecular hydrogen bonding patterns found for glycine. Amino amide derivatives ^{40,41}, including valinamide⁴², are stabilized by an intramolecular hydrogen bond from the amide to the amino nitrogen. Only one conformation, with the isopropyl side group oriented with $\chi_1 = -62^\circ$, was observed for valinamide. No tunneling splittings arising from methyl internal rotation on the isopropyl side chain were observed for neutral valine or valinamide.

Biomimetic molecules with methyl rotors may have rotational transitions split into *A* and *E* components arising from methyl internal rotation tunneling⁴³. Assignment of these tunneling components can be used to find the torsional barrier and rotor orientation^{10,44}. Analysis of the torsion-rotation splittings in microwave spectra can be used to determine the angles that the methyl rotors make with respect to the principal inertial axes. This approach was used to identify conformational structures of *N*-acetyl-alanine-*N*-methylamide ⁴⁵, ethylacetamidoacetate ⁴⁶, *N*-acetylglycine ⁴⁷, and *N*-acetyl alanine methyl ester ⁴⁸. Torsion-rotation spectra were also assigned for a series of methyl indoles, substituted in different positions around the rings¹⁰. Assignment and fitting of the *A*- and *E*-state transitions were used to find the *V*₃ barriers, which range from 125.1 cm⁻¹ to 436.7 cm⁻¹ depending on location and the local electronic environment.

Amino acid methyl esters provide an opportunity to explore both the structural impacts of ester derivatization as well as the rotor barrier and orientation from analysis of methyl internal rotation tunneling. Two rotational-spectroscopic investigations of amino acid methyl esters have been reported previously: glycine methyl ester ⁴⁹ and *N*-acetyl alanine methyl ester ⁴⁸. The rotational spectrum of glycine methyl ester arises from a single conformation with a bifurcated hydrogen bond from the amine to the carbonyl oxygen⁴⁹, similar to the lowest energy conformation of glycine⁷. Internal-rotation tunneling splittings were assigned, and the methyl *V*₃ barrier was found to be 411.66(10) cm⁻¹. Tunneling from two methyl rotors complicates the rotational spectrum of *N*-acetyl alanine methyl ester, splitting each rotational transition into components arising from the *AA*, *AE*, and *EA* tunneling states. The barrier to methyl internal rotation was found to be 396.46(7) cm⁻¹ for the methoxy methyl and 64.96(4) cm⁻¹ for the acetyl methyl group.

The rotational spectrum of valine methyl ester (ValOMe; see Figure 4.1 for atom-numbering scheme) is reported here as part of a larger investigation of biomolecule esters to understand the

conformational preferences of ester derivatives and strengthen our understanding of the torsional barriers of methyl esters, which may be sensitive to the electronic environment of the methyl rotor.



Figure 4.1. The numbering scheme for (A) ValOMe and comparison to the structures of (B) neutral valine, and (C) valinamide.

4.2 Experimental and Computational Methods

Valine methyl ester hydrochloride (99% pure) was purchased from Sigma-Aldrich, dissolved in 1M aqueous NaHCO₃ solution, and extracted using ethyl acetate to isolate pure ValOMe. Sample purity was confirmed by NMR spectroscopy.

Rotational spectra of ValOMe conformers were recorded in the 9-18 GHz frequency range using mini cavity-based Fourier-transform microwave spectrometer ^{32,33} described in detail elsewhere³¹. Sample was placed in a reservoir nozzle⁵⁰ and heated to 45°C using a Watlow band heater and an Omega CN8201 temperature controller to maintain an optimum vapor pressure of the sample. Argon, with a 1-atm backing pressure, was used as the carrier gas, and the gas mixture was expanded into the vacuum chamber during an 800 µs long pulse of a Series 9 General Valve. Each gas pulse is followed by a 0.2 µs microwave irradiation pulse, and the detection circuit is closed after an additional 2 µs delay. The induced signal is digitized at 100 MHz for 400 µs. A custom LabVIEW program controls the spectrometer operations, performs signal averaging, transforms the time domain signals into frequency domain by a fast Fourier-transform algorithm, and saves data.

The supersonic expansion is coaxial along the cavity resonator axis ⁵¹. Due to this parallel arrangement, each transition's signal is split into Doppler doublets centered at the transition frequency. The Doppler splitting depends on frequency, ranging from 35 kHz near 9 GHz to 70 kHz near 18 GHz. The average full width at half maximum of each component of the doublet is 13 kHz. The digital frequency resolution of the spectrometer is 2.5 kHz, and the signal quality depends on the number of shots averaged for the scan. Well-resolved portions of the microwave spectra of conformers I and II of ValOMe are shown in Figures 2 and 3 respectively. Despite the

extensive splitting, the Doppler components are not significantly broadened compared to unsplit spectra, and we estimate the experimental uncertainties for most line centers to be 5 kHz.



Figure 4.2. Portion of the microwave spectrum of ValOMe (Conformer I) showing (*A*) and (*E*) tunneling state components and nuclear quadrupole hyperfine components of the 5_{23} - 4_{13} rotational transition with 2000 shots averaged.



Figure 4.3. Portion of the microwave spectrum of ValOMe (Conformer II) showing (*A*) and (*E*) tunneling state components and nuclear quadrupole hyperfine components of the 5_{23} - 4_{13} rotational transition with 2000 shots averaged.

Density functional theory (DFT) calculations were performed using the GAUSSIAN 16 program 371 running on the Owens cluster at the Ohio Supercomputer Center. Full geometry optimizations of different conformers of the molecule were carried out using the ω B97XD hybrid density functional 52 with the 6-311++G(d, p) basis set 53 . Estimated methyl barrier heights for each of the C-CH₃ rotors and the O-CH₃ rotor for each conformer were calculated using relaxed potential energy scans at the ω B97XD/6-311++G(d, p) level, fixing the rotor dihedral angle in 30° steps while optimizing all remaining parameters. Several older programs, including RRFIT (rigid-rotor fitting), ZFAP (rotational and centrifugal distortion fitting) 54 , and QUAD2I (an updated version of QUAD2 for nuclear quadrupole hyperfine fitting), were used for initial fits of the spectra. Global fits of the rotational constants, centrifugal distortion constants, nuclear quadrupole coupling constants, and *A*- and *E*- tunneling states were performed using XIAM 55

4.3 Results

The DFT calculations identified ten different conformational minima of ValOMe, but only the four lowest energy conformers (shown in Figure 4) were considered further as they were within 15 kJ mol⁻¹ of the global minimum (Table S1). Table 1 provides the relative energies, theoretically calculated rotational constants, dipole moment projections, and H-bond distances of the four lowest-energy conformers. Conformers I and II each have a bifurcated hydrogen bonding network from the amino nitrogen protons to the carbonyl oxygen – similar to the hydrogen-bonding network of the lowest-energy conformations of neutral amino acids. Conformer II is 2.08 kJ mol⁻¹ higher in energy than conformer I and differs principally in the orientation of the isopropyl side chain ($\chi_1 = -64.0^\circ$ in conformer I and = 173.6° for conformer II). The carboxylic-acid-to-aminonitrogen hydrogen bonding network, observed as a higher-energy configuration of the amino acids, is blocked by the ester derivatization. Instead, conformers III and IV each have a single intramolecular H-bond from one of the amino hydrogens to the methoxy oxygen, and these conformers are calculated to be much higher in energy.

Rotational spectra were measured and assigned to the two lowest-energy conformers of ValOMe. Both spectra were rich with transitions allowed by all 3 dipole selection rules (*a*-, *b*-, and *c*-types). 231 nuclear quadrupole hyperfine components were fit to 44 rotational transition center frequencies and the nuclear quadrupole coupling constants: $\chi_{aa} = -4.187(7)$ MHz and $\chi_{bb} - \chi_{cc} = 1.269(5)$ MHz for conformer I. The frequencies of all resolved transitions for conformer I are available in the Supporting Information, Table S4. An additional 15 rotational transitions were measured for conformer I, but overlapping hyperfine components could not be resolved and assigned. Among the 59 rotational transitions of conformer I, there were 23 *a*-type, 20 *b*-type, and 16 *c*-type transitions. For conformer II, 47 rotational transitions with 264 resolved nuclear hyperfine components were fit to the nuclear quadrupole coupling constants: $\chi_{aa} = -4.187(7)$ MHz and $\chi_{bb} - \chi_{cc} = 1.518(6)$ MHz. The frequencies of the resolved transitions for conformer II are available in the Supporting Information, Table S5.

Parameter	Conformer I	Conformer II	Conformer III	Conformer IV	
$\Delta E / \text{kJ mol}^{-1}$	0	2.08	9.97	14.17	
A/MHz	2550.9	2536.6	3040.3	2993.3	
<i>B</i> /MHz	1038.9	1099.6	989.4	1004.7	
C/MHz	938.4	897.7	883.7	824.8	
μ_a/D	-0.64	0.33	-0.55	0.19	
χ_{aa} / MHz	-4.811	-4.751			
$\chi_{bb} - \chi_{cc} / \mathrm{MHz}$	1.097	1.415			
$\mu_b/{ m D}$	0.21	-0.51	-0.44	-0.49	
μ_c/\mathbf{D}	0.36	-0.42	-0.46	-0.82	
χ_1	-64.0°	173.6°	-57.3°	-166.7°	
H-bond/Å					
a	2.506	2.585	2.208	2.393	
b	3.022	3.195			

Table 4.1. Theoretically Calculated Spectroscopic Parameters for ValOMe at ω B97XD/6-311++G(d,p) level of theory.



Figure 4.4. Structures of the lowest-energy model conformers of ValOMe at the ω B97XD/6-311++G(d,p) level. χ_1 is the dihedral angle $H^{\beta} - C^{\beta} - C^{\alpha} - H^{\alpha}$.

Final fitting of the assigned transitions for each conformer was carried out using XIAM and the *A*-reduction Hamiltonian. Each spectrum was fit to rotational constants, nuclear quadrupole coupling constants, quartic centrifugal distortion constants (excluding Δ_K , which was fixed to 0 in both fits), the barrier to internal rotation (V₃), ε and δ (polar-coordinate angles describing the orientation of the internal rotor axis ⁵⁵). The higher order parameters D_{pi2J} , D_{pi2K} , and D_{pi2-} did not converge in preliminary fits of the spectra and were fixed to zero; the rotor moment of inertia (I_α) was fixed to 3.2 amu Å². The fits have RMS errors of 5.6 kHz (conformer I) and 4.8 kHz (conformer II), and the best fit values of these constants and the torsional rotor-axis angles ($\angle(i, a), \angle(i, b)$, and $\angle(i, c)$) are given in Table 2. Table 2 also compares the experimentally fit and theoretical model parameters of both conformers. The best-fit values of the rotor-axis angles ($\angle(i, a), \angle(i, b)$, and $\angle(i, c)$) for both conformers are in good agreement with the rotor angles calculated from the model structures. The experimental and model rotational constants are in excellent agreement for each conformer, and the DFT model structures predicted most rotational transitions within 10 MHz.

The relaxed potential scans were used to estimate the barriers to methyl internal rotation for the isopropyl methyl groups and the methoxy methyl group. For conformer I, the calculated barriers for the isopropyl methyl groups and the methoxy methyl group were 1151.8 cm⁻¹, 1077.7 cm⁻¹, and 350.8 cm⁻¹ respectively. The barriers to internal rotation were found to be 1057.0 cm⁻¹ and 1183.7 cm⁻¹ for the isopropyl methyl groups and 355.7 cm⁻¹ for the methoxy methyl group of conformer II. The potential energy scans for methyl barriers are shown in figures .

	Conformer I		Conformer II			
Parameter	Experimental	Theoretical	Experimental	Theoretical		
A/MHz	2552.0144(5)	2550.9	2544.2839(3)	2536.6		
<i>B</i> /MHz	1041.8216(3)	1038.9	1092.3654(15)	1099.6		
C/MHz	938.5489(21)	938.4	896.3131(12)	897.7		
⊿ _J /kHz	0.1486(23)		0.166(11)			
Δ_{JK}/kHz	0.165(13)		-0.179(9)			
Δ_k/kHz	0.00^{a}		0.00^{a}			
δ_{J}/kHz	0.037(12)	0.0588(5)				
$\delta_{\it K}$ /kHz	0.11(8)		0.228(24)			
χ_{aa} / MHz	-4.187(7)		-4.187(7)			
$\chi_{bb} - \chi_{cc} / \mathrm{MHz}$	1.269(5)		1.518(6)			
$V_3 /{ m cm}^{-1}$	401.64(19)	350.8	409.74(16)	355.7		
$\angle(i, a)$ /°	26.5(3)	20.4	20.4(3)	21.2		
$\angle(i, b)$ /°	77.5(6)	83.0	77.2(5)	79.1		
$\angle(i, c) /^{\circ}$	67.0(5)	70.9	74.4(5)	72.1		
N^b	44		47			
No. A lines ^c	126		141			
No. E lines ^{c}	105		123			
⊿v _{rms} /kHz	4.8		4.6			

Table 4.2. Comparison between experimentally determined and theoretically calculated rotational spectroscopic parameters for ValOMe.

^{*a*}Parameter fixed to 0.00 in fitting. ^{*b*}Number of rotational transitions in the fit. ^{*c*}Including hyperfine components

4.4 Discussion

The two experimentally measured spectra were assigned to the lowest two energy theoretical structures because of the excellent agreement between the model and experimentally determined rotational constants in Table 2; the experimental values of A are within 0.4% of the model values and B and C are within 0.7%, and 0.2%, respectively. The DFT-calculated values for the nuclear quadrupole coupling constant, $\chi_{bb} - \chi_{cc}$, for conformers I and II are also consistent with the assignment of the spectra to conformers I and II; see Table 2. Second moments of inertia, Paa, Pbb, and Pcc, provide a more robust means of assignment. The second moment along the aaxis is given by $P_{aa} = \sum m_i a_i^2 = \frac{1}{2} (-I_a + I_b + I_c)$ where the m_i are the atomic masses, a_i are the atomic coordinates along the *a* inertial axis, and I_a, I_b, and I_c are the principal moments of inertia; there are similar expressions for P_{bb} and P_{cc} ⁵⁶. Table 3 compares the second moments determined using the second equality and moments of inertia derived from the experimental rotational constants $(B = h/8\pi^2 I_b)$ with the second moments calculated from the coordinates of the theoretical structures using the first equality. The theoretical models for conformers I and II are easily distinguished by their values for P_{bb} and P_{cc}, and the experimentally determined values for these second moments unambiguously confirm the assignment. Because the theoretical structures reproduce the experimental spectroscopic constants so well, the theoretical structures can be considered as the experimental structures, and no further structural fitting is necessary.

	Conformer I		Conformer II	Conformer II			
Parameter	Experimental	Theoretical	Experimental	Theoretical			
P_{aa} / amu Å ²	412.764	413.429	413.928	411.660			
P_{bb} / amu Å 2	125.704	125.108	149.914	151.281			
P_{cc} / amu Å ²	72.327	73.001	48.719	47.954			

Table 4.3. Comparison of model and experimental values of the second moments of inertia for conformers I and II.

Conformers I and II were identified as the two lowest-energy conformations in an early computational conformational analysis at the HF/4-21G level, but that model predicts a much higher-energy (6.3 kJ mol⁻¹) for conformer II ⁵⁷. A more recent conformational analysis of ValOMe using B3LYP/aug-cc-pVDZ calculations identified conformers I and II as the most stable conformers (I_c and I_b) of ValOMe ⁵⁸. The ${}^{3}J_{HH}$ spin-spin coupling constants were calculated for each conformer using the IEF-PCM model. The good agreement with experimental ¹H data indicated that these two conformations predominate the conformational distribution, both isolated in the gas phase and in various solvents⁵⁸.

The principal difference between the two lowest-energy ValOMe conformers is the orientation of the side chain. The dihedral angle χ_1 (H^{β} – C^{β} – C^{α} – H^{α}) describes the orientation of the isopropyl group. χ_1 = -64.0° for conformer I and χ_1 = 173.6° for conformer II. Newman projections about χ_1 for the four lowest energy conformations are given in Figure 5. Interestingly, the χ_1 = -64° orientation is the only configuration that has been detected previously for neutral value ⁹ and valuamide ⁴² conformers.



Figure 4.5. Newman projections through the dihedral angle (χ_1) of the four lowest energy conformers from DFT calculations.



Dihedral angle (°)

Figure 4.6. Relaxed potential scans for isopropyl and methoxy methyl groups of conformer I along the given dihedral angle.



Figure 4.7. Relaxed potential scans for isopropyl and methoxy methyl groups of conformer II along the given dihedral angle.

As noted above, both lowest energy conformers of ValOMe are stabilized by the same network of intramolecular hydrogen bonds involving the amino hydrogens to the carbonyl oxygen. The H-bond distances in the two conformers are very similar, approximately 2.55 Å and 3.2 Å (see Table 1), with N...O distances of 2.818 Å and 2.902 Å, which fall within the sum of their van der Waals radii ^{59,60}. The N...H...O angles, however, are 97° for H_a and 64 – 69° for H_b, indicating weak hydrogen-bonding interactions using the criteria described by Nagy ⁶¹. There are additional close CH ... N and CH ... O interactions in both conformers. CH ... N and CH ... O distances are less than 3.3 Å are given in Table 4 for each conformer. Some of these interaction distances are less than 2.7 Å, suggesting that these interactions are significant. The relative energies of the different conformers, therefore, is dependent on the aggregate of all H-bonding and other weak interactions.

Table 4.4. Other Weak Interaction Distances in ValOMe Conformers.

Conformer I		Conformer II		
$H_{10} \dots N$	3.299	$H_{10} \dots O_2$	3.064	
$H_{12} \ldots N$	2.716	$H_{12} \dots O_9$	3.064	
H ₁₆ N	2.685	$H_{13} \dots N$	2.619	
		H ₁₃ O ₉	2.973	
		$H_{14} \ldots N$	2.574	

The theoretical barrier heights for methoxy methyl rotor and two methyl rotors of the isopropyl group were calculated at the ω B97XD/6- 311++G(d,p) level of theory. The barriers for the methoxy rotors are much lower than the methyl-rotor barriers of the isopropyl group. The methoxy barriers were calculated to be 350.8 cm⁻¹ for conformer I and 355.7 cm⁻¹ for conformer II. The experimentally fit values for the *V*₃ barriers are 401.64(19) cm⁻¹ and 409.74(16) cm⁻¹ for conformers I and II; see Table 2. While the experimental barriers are 50 – 60 cm⁻¹ larger than the ω B97XD models, the models do correctly predict conformer II methoxy rotor is larger than that

for conformer I. The similarities of the barriers and rotor-axis angles for the two conformers, however, precludes their use for assigning the ValOMe spectra to the two lowest-energy conformers. The experimental values of the V₃ barriers for ValOMe are within the range of values from previous studies of biomolecule esters: glycine methyl ester ⁴⁹ (411.66(10) cm⁻¹) and N-acetyl alanine methyl ester ⁴⁸ (396.46(7) cm⁻¹).

The methyl-rotor barriers of the isopropyl group were calculated to be 1151.8 and 1077.7 cm⁻¹ for conformer I and 1183.7 and 1057.0 cm⁻¹ for conformer II. Barriers over 1000 cm⁻¹ may not have resolved tunneling splittings in the microwave spectrum, and tunneling splittings arising from the isopropyl group methyl rotors were not resolved in the spectra of the two conformers of ValOMe. No tunneling splittings from the isopropyl methyl groups were resolved in the spectra of neutral valine ⁹ and valinamide ⁴².

4.5 Conclusion

Rotational spectrum has been recorded for valine methyl ester in the 9-18 GHz frequency range. The spectra of ValOMe were assigned to the two lowest-energy theoretical structures calculated from the ω B97XD/6- 311++G(d,p) level of theory, and XIAM was employed to fit the splittings arising from ¹⁴N nuclear quadrupole coupling and internal rotation of the methyl rotor. The two lowest-energy structures have similar intramolecular H-bonding networks; the principal difference between these conformations is the orientation of the isopropyl side chain. The experimentally determined barriers to methyl internal rotation of ValOMe conformer I and conformer II are 401.64(19) cm⁻¹ and 409.74(16) cm⁻¹, respectively. These values were found to be comparable to the barriers for methyl internal rotation in related biomolecule ester species.

4.6 Optimized Molecular Coordinates for ValOMe conformers

4.6.1 Cartesian coordinates (Å) of ω B97XD/6- 311++G(d,p) optimized structures of the lowest energy conformers (I and II) of Valine Methyl Ester (ValOMe).

Atom Number	Atom	а	b	c		
1	С	1.465928	-0.537150	1.525982		
2	С	1.279017	-0.711013	0.017699		
3	Н	0.512213	-0.432469	2.051804		
4	Н	1.985172	-1.402751	1.945007		
5	Н	2.073202	0.346756	1.742704		
6	С	0.450514	0.445240	-0.596569		
7	С	-0.982090	0.388477	-0.071752		
8	С	2.624779	-0.833646	-0.693799		
9	Н	0.708350	-1.629127	-0.157427		
10	Н	2.491666	-1.034718	-1.761005		
11	Н	3.210767	-1.652569	-0.267460		
12	Н	3.194444	0.092768	-0.591542		
13	0	-1.643775	-0.670528	-0.556056		
14	С	-2.982802	-0.842870	-0.088676		
15	Н	-2.995611	-0.971734	0.994911		
16	Н	-3.594017	0.021932	-0.351775		
17	н	-3.354343	-1.737532	-0.583439		
18	0	-1.468129	1.178189	0.696669		
19	N	1.079631	1.732985	-0.371034		
20	Н	0.400795	0.266761	-1.675280		
21	Н	0.698400	2.429005	-0.999397		
22	Н	0.874696	2.057272	0.567924		

Table 4.5. Optimized molecular structure for conformer I of ValOMe. The coordinates of methyl rotor are highlighted.

Atom Number	Atom	a	b	с	
1	С	0.928734	-1.834618	0.342027	
2	С	1.465520	-0.402388	0.338278	
3	Н	0.766059	-2.194265	-0.679169	
4	Н	1.646930	-2.503347	0.822904	
5	Н	-0.016503	-1.922739	0.884593	
6	С	0.522591	0.575564	-0.397737	
7	С	-0.900793	0.397252	0.117544	
8	С	2.863581	-0.346654	-0.274774	
9	Н	1.522462	-0.053958	1.377760	
10	Н	3.232364	0.678703	-0.314222	
11	Н	3.562492	-0.950946	0.309953	
12	Н	2.849507	-0.745856	-1.295261	
13	0	-1.659310	-0.322548	-0.718535	
14	С	-2.991743	-0.593557	-0.278627	
15	H	-3.451269	-1.176988	-1.073330	
16	H	-2.976650	-1.161625	0.653314	
17	H	-3.539135	0.336729	-0.119521	
18	0	-1.303901	0.843267	1.162045	
19	Ν	0.991781	1.943433	-0.230992	
20	Н	0.531434	0.329067	-1.463689	
21	Н	0.822634	2.245182	0.723312	
22	Н	0.485558	2.576661	-0.838050	

Table 4.6. Optimized molecular structure for conformer II of ValOMe. The coordinates of methyl rotor are highlighted.

4.7 Assigned transition frequencies for conformer I and II of ValOMe.

	<i>K'</i>	<u> </u>	<u>F</u>	1.7. As	signed	transi	$\frac{\text{tion free}}{F''}$	equencies for	<u>conformer I.</u>	Fobs	F obs-calc
J	na	n _c	1	J	Na	Λ _C	1	$(\mathbf{M}\mathbf{U}_{\mathbf{z}})$			
	4				4						
5	1	5	5	4	1	4	4	9622.812	0.005	9622.803	0.003
			4				3	9622.865	0.006	9622.848	0.006
			6				5	9622.897	0.001		
5	0	5	4	4	0	4	3	9803.002	0.001	9802.960	0.003
			6				5	9803.080	0.003	9803.030	0.003
			5				4	9803.098	0.004	9803.044	0.006
5	2	4	5	4	2	3	4	9890.214	0.004	9890.993	0.010
			6				5	9890.504	0.007	9891.280	0.006
			4				3	9890.538	0.002	9891.314	0.011
5	3	3	5	4	3	2	4	9917.273	0.009	9918.791	0.002
			6				5	9917.926	0.006	9919.443	0.002
			4				3	9918.082	0.009	9919.598	0.001
5	3	2	6	4	3	1	5	9921.240	0.007		
			4				3	9921.402	0.006		
5	2	3	5	4	2	2	4	9988.883	0.005	9988.092	0.008
			6				5	9989.214	0.005	9988.421	0.005
4	1	3	3	3	0	3	2	10011.127	0.001	10011.016	0.001
			4				3	10011.187	0.010	10011.061	0.003
			5				8	10011.236	0.002	10011.127	0.002
5	1	4	5	4	1	3	4	10135.810	0.001	10135.778	0.001
			4				3	10135.841	0.006	10135.802	0.001
			6				5	10135.895	0.001	10135.852	0.010
3	2	2	2	2	1	1	1	10471.277	0.003	10467.036	0.000
			4				3	10471.765	0.004	10467.522	0.001
			3				2	10472.640	0.001	10468.394	0.001
3	2	1	2	2	1	1	1	10496.801	0.004	10500.011	0.003

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			4				3	10497.272	0.002	10500.484	0.001
			3				2	10498.110	0.002	10501.324	0.001
5	1	5	4	4	0	4	3	10731.284	0.005	10731.116	0.006
			6				5	10731.408	0.002	10731.244	0.002
			5				4	10731.701	0.001	10731.536	0.002
3	2	2	2	2	1	2	1	10781.395	0.001	10777.339	0.002
			4				3	10781.663	0.007	10777.597	0.002
			3				2	10782.126	0.002	10778.060	0.003
6	0	6	6	5	1	5	5	10786.868	0.000	10786.922	0.010
			7				6	10787.121	0.004	10787.173	0.007
			5				4	10787.121	0.000	10787.173	0.012
3	2	1	2	2	1	2	1	10806.920	0.000	10810.307	0.006
			4				3	10807.174	0.008	10810.562	0.002
			3				2	10807.595	0.002	10810.993	0.000
6	1	6	6	5	1	5	5	11535.628	0.002		
			5				4	11535.640	0.000	11535.614	0.002
			7				6	11535.673	0.003	11535.640	0.005
6	0	6	5	5	0	5	4	11715.398	0.001		
			7				6	11715.456	0.006		
			6				5	11715483	0.006	11715.405	0.010
6	2	5	6	5	2	4	5	11859.013	0.001		
			5				4	11859.181	0.001		
6	3	4	6	5	3	3	5	11905.708	0.006	11908.933	0.006
			7				6	11906.087	0.011	11909.316	0.004
			5				4	11906.151	0.006	11909.377	0.006
6	3	3	6	5	3	2	5	11914.490	0.006	11911.239	0.006
			7				6	11914.878	0.007	11911.626	0.006
			5				4	11914.940	0.005	11911.688	0.008
6	1	5	5	5	1	4	4	12146.393	0.003	12146.355	0.000
			6				5	12146.393	0.001	12146.355	0.002
			7				6	12146.439	0.004	12146.393	0.007

5	1	4	5	4	0	4	4	12275.930	0.001	12275.819	0.004
			4				3	12276.024	0.007	12275.908	0.009
			6				5	12276.080	0.006	12275.966	0.006
4	2	3	3	3	1	2	2	12296.247	0.002	12294.375	0.002
			5				4	12296.540	0.002	12294.667	0.001
			4				3	12297.229	0.000	12295.354	0.002
4	2	2	3	3	1	2	2	12372.207	0.002	12373.137	0.005
			5				4	12372.476	0.003	12373.406	0.001
			4				3	12373.090	0.002	12374.020	0.001
6	1	6	5	5	0	5	4			12463.773	0.004
			7				6	12463.998	0.005	12463.855	0.000
			6				5	12464.233	0.001	12464.090	0.004
7	0	7	7	6	1	6	6	12860.930	0.004	12860.926	0.004
			6				5	12861.105	0.007	12861.105	0.001
			8				7	12861.105	0.005	12861.105	0.004
4	2	3	3	3	1	3	2	12915.945	0.003	12914.168	0.003
			5				4	12916.099	0.007	12914.315	0.000
			4				3	12916.352	0.002	12914.574	0.002
4	2	2	3	3	1	3	2	12991.901	0.002	12992.924	0.005
			5				4	12992.040	0.011	12993.059	0.004
			4				3	12992.220	0.002	12993.241	0.005
7	1	7	7	6	1	6	6	13443.246	0.005		
			6				5	13443.246	0.004		
			8				7	13443.265	0.000	13443.232	0.008
7	0	7	6	6	0	6	5	13609.621	0.004	13609.528	0.006
			8				7	13609.660	0.007	13609.569	0.002
			7				6	13609.690	0.007	13609.595	0.006
7	2	6	7	6	2	5	6	13822.723	0.001		
			6				5	13822.817	0.001		
			8				7	13822.829	0.003		
7	3	5	7	6	3	4	6	13895.347	0.004	13899.466	0.011

			8				7	13895.592	0.005	13899.710	0.009
			6				5	13895.616	0.004	13899.736	0.013
7	3	4	7	6	3	3	6	13914.907	0.004	13910.763	0.013
			8				7	13915.161	0.004	13911.016	0.011
			6				5	13915.185	0.003	13911.042	0.014
5	2	4	4	4	1	3	3	14069.535	0.003	14068.465	0.005
			6				5	14069.722	0.005	14068.651	0.002
			5				4	14070.282	0.002	14069.208	0.002
7	1	6	6	6	1	5	5	14146.432	0.009		
			8				7	14146.462	0.014		
7	1	7	6	6	0	6	5	14191.760	0.000	14191.643	0.004
			8				7	14191.824	0.006	14191.700	0.006
			7				6	14191.998	0.001	14191.888	0.002
5	2	3	4	4	1	3	3	14244.206	0.000	14244.365	0.000
			6				5	14244.367	0.005	14244.531	0.001
			5				4	14244.815	0.002	14244.978	0.001
6	1	5	6	5	0	5	5	14619.233	0.006	14619.115	0.006
			5				4	14619.424	0.003	14619.312	0.002
			7				6	14619.440	0.008	14619.329	0.003
5	2	4	4	4	1	4	3	15101.306	0.009	15100.294	0.011
			6				5	15101.400	0.008	15100.387	0.004
			5				4	15101.518	0.001	15100.509	0.002
5	2	3	4	4	1	4	3	15275.980	0.003		
			6				5	15276.042	0.004		
			5				4	15276.050	0.001	15276.271	0.007
8	1	8	8	7	1	7	7	15345.693	0.005		
			7				6	15345.697	0.005		
			9				8	15345.711	0.000		
8	0	8	7	7	0	7	6	15489.705	0.002		
			9				8	15489.734	0.000		
			8				7	15489.763	0.000	15489.648	0.005

6	2	5	5	5	1	4	4	15792.873	0.12	15792.112	0.002
			7				6	15793.009	0.007	15792.248	0.006
			6				5	15793.487	0.000	15792.719	0.006
6	2	4	7	5	1	4	6	16134.194	0.003	16134.070	0.003
			6				5	16134.509	0.004	16134.386	0.003
6	2	5	5	5	1	5	4	17337.623	0.014	17336.903	0.014
			7				6	17337.685	0.005	17336.965	0.005
			6				5	17337.720	0.006	17336.999	0.006

J'	K'a	K' _c	F'	J''	<i>K</i> _{<i>a</i>} ^{''}	<i>K</i> _c ''	<i>F''</i>	A obs	A obs-calc	E obs	E obs-calc
								(MHz)	(MHz)	(MHz)	(MHz)
5	1	5	5	4	1	4	4	9382.773	0.007	9382.767	0.003
			4				3	9382.808	0.003	9382.803	0.001
			6				5	9382.847	0.000	9382.838	0.001
5	0	5	4	4	0	4	3	9613.823	0.005	9613.784	0.003
			6				5	9613.902	0.004	9613.864	0.001
			5				4	9613.961	0.003	9613.921	0.004
5	2	4	5	4	2	3	4	9901.962	0.001	9902.146	0.001
			6				5	9902.244	0.002	9902.425	0.003
			4				3	9902.279	0.005	9902.459	0.003
5	3	3	5	4	3	2	4	9995.820	0.001		
			6				5	9996.475	0.002		
			4				3	999.632	0.004		
5	3	2	5	4	3	1	4	10018.523	0.002		
			6				5	10019.191	0.003		
			4				3	10019.340	0.015		
5	1	5	4	4	0	4	3	10175.206	0.001	10175.085	0.006
			6				5	10175.330	0.002	10175.206	0.006
			5				4	10175.595	0.003	10175.466	0.002
5	2	3	5	4	2	2	4	10230.888	0.003	10230.698	0.000
			6				5	10231.244	0.005	10231.071	0.015
			4				3	10231.281	0.001	10231.091	0.002
5	1	4	4	4	1	3	3	10340.249	0.001	10340.215	0.008
			5				4	10340.249	0.004	10340.215	0.010
			6				5	10340.315	0.000	10340.282	0.005
3	2	1	2	2	1	1	1	10413.029	0.004		
			4				3	10413.516	0.008		
			3				2	10414.334	0.007		

 Table 4.8. Assigned transition frequencies for conformer II.

4	1	3	4	3	0	3	3	10523.726	0.007	10523.608	0.014
			3				2	10523.781	0.009	10523.665	0.019
			5				4	10523.856	0.001	10523.745	0.014
6	0	6	6	5	1	5	5	10849.514	0.006	10849.553	0.003
			5				4	10849.669	0.006	10849.706	0.001
			7				6	10849.689	0.007	10849.710	0.002
3	2	2	2	2	1	2	1	10909.863	0.002	10908.464	0.003
			4				3	10910.100	0.003	10908.708	0.003
			3				2	10910.533	0.001	10909.131	0.002
3	2	1	2	2	1	2	1	11001.551	0.000	11002.228	0.001
			4				3	11001.758	0.003	11002.438	0.005
			3				2	11002.082	0.004	11002.765	0.000
6	1	6	6	5	1	5	5	11223.319	0.002	11223.310	0.000
			5				4	11223.319	0.002	11223.310	0.003
			7				6	11223.358	0.005	11223.348	0.006
6	0	6	5	5	0	5	4	11411.049	0.005	11410.994	0.003
			7				6	11411.103	0.002	11411.049	0.001
			6				5	11411.156	0.002	11411.103	0.006
6	1	6	5	5	0	5	4	11784.709	0.006	11784.620	0.014
			7				6	11784.785	0.009	11784.681	0.003
			6				5	11784.956	0.001	11784.857	0.000
6	2	5	6	5	2	4	5	11848.941	0.002	11848.999	0.004
			5				4	11849.095	0.002	11849.153	0.004
			7				6	11849.095	0.005	11849.153	0.001
6	4	3	6	5	4	2	5	11993.915	0.002	11994.871	0.001
			7				6	11994.591	0.004	11995.546	0.000
			5				4	11994.732	0.002	11995.684	0.005
6	3	4	6	5	3	3	5	12005.670	0.001	12009.285	0.001
			7				6	12006.055	0.003	12009.665	0.003
			5				4	12006.118	0.001	12009.728	0.002
4	2	3	3	3	1	2	2	12013.915	0.000	12013.137	0.001

			5				4	12014.222	0.001	12013.443	0.001
			4				3	12014.944	0.002	12014.166	0.002
6	3	3	6	5	3	2	5	12064.815	0.001	12061.189	0.000
			7				6	12065.223	0.003	12061.599	0.003
			5				4	12065.289	0.001	12061.668	0.004
4	2	2	3	3	1	2	2	12281.785	0.002	12281.721	0.002
			5				4	12282.045	0.000	12281.983	0.002
			4				3	12282.599	0.000	12282.537	0.003
6	1	5	5	5	1	4	4	12340.307	0.006	12340.256	0.008
			6				5	12340.343	0.006	12340.294	0.005
			7				6	12340.357	0.006		
6	2	4	6	5	2	3	5	12367.797	0.001	12367.727	0.000
			5				4	12368.016	0.001	12367.947	0.001
			7				6	12368.016	0.001	12367.947	0.001
7	1	7	6	6	1	6	5	13051.258	0.003	13051.258	0.001
			7				6	13051.266	0.003	13051.266	0.001
			8				7	13051.282	0.002	13051.282	0.002
4	2	3	3	3	1	3	2	13189.378	0.006	13188.647	0.000
			5				4	13189.516	0.002	13188.777	0.000
			4				3	13189.732	0.002	13188.992	0.005
7	1	7	6	6	0	6	5	13424.912	0.001		
			8				7	13424.964	0.008		
			7				6	13425.076	0.006		
7	2	6	7	6	2	5	6	13778.487	0.007	13778.487	0.011
			6				5	13778.577	0.003	13778.577	0.000
			8				7	13778.592	0.006	13778.592	0.003
7	3	5	7	6	3	4	6	14014.017	0.001		
			8				7	14014.263	0.000		
			6				5	14014.286	0.001		
7	3	4	7	6	3	3	6	14141.844	0.000		
			8				7	14142.125	0.000		

			6				5	14142.152	0.001		
5	2	3	4	4	1	3	3	14206.895	0.000	14206.665	0.000
			6				5	14207.039	0.003	14206.811	0.002
			5				4	14207.385	0.003	14207.158	0.001
7	1	6	6	6	1	5	5	14295.430	0.002	14295.373	0.001
			8				7	14295.468	0.001	14295.414	0.000
			7				6	14295.487	0.003	14295.430	0.000
8	0	8	8	7	1	7	7	14724.708	0.011	14724.575	0.005
			7				6	14724.749	0.005	14724.614	0.001
			9				8	14724.768	0.001	14724.634	0.006
8	1	8	7	7	1	7	6	14868.608	0.003	14868.627	0.002
			8				7	14868.627	0.003	14868.648	0.006
			9				8	14868.627	0.002	14868.648	0.000
8	0	8	7	7	0	7	6	14961.164	0.002	14961.057	0.002
			9				8	14961.188	0.002	14961.085	0.002
			8				7	14961.218	0.000	14961.111	0.001
8	1	8	7	7	0	7	6	15105.016	0.007	15105.075	0.003
			9				8	15105.047	0.005	15105.103	0.001
			8				7	15105.124	0.002	15105.179	0.007
6	2	5	5	5	1	4	4	15118.867	0.001	15118.375	0.002
			7				6	15119.002	0.000	15118.503	0.006
			6				5	15119.499	0.002	15119.002	0.002
5	2	4	4	4	1	4	3	15563.033	0.008	15562.484	0.005
			6				5	15563.106	0.001	15562.557	0.002
			5				4	15563.191	0.003	15562.644	0.008
8	2	7	8	7	2	6	7	15688.973	0.010	15688.938	0.004
			7				6	15689.009	0.007	15688.973	0.008
			9				8	15689.022	0.006	15688.991	0.004
8	3	6	8	7	3	5	7	16016.638	0.001	16017.136	0.005
			9				8	16016.800	0.001	16017.297	0.003
			7				6	16016.805	0.001	16017.303	0.003

1	7	7	7	1	6	6	16196.483	0.001	16196.400	0.008
		9				8	16196.518	0.004	16196.430	0.010
		8				7	16196.557	0.004	16196.483	0.004
2	4	5	5	1	4	4	16234.658	0.003	16234.389	0.001
		7				6	16234.743	0.002	16234.475	0.001
		6				5	16234.928	0.003	16234.658	0.003
3	5	8	7	3	4	7	16256.327	0.002	16255.831	0.005
		9				8	16256.536	0.000	16256.042	0.005
		7				6	16256.548	0.002	16256.052	0.005
2	6	8	7	2	5	7	16619.643	0.001	16619.615	0.002
		7				6	16619.711	0.001	16619.672	0.009
		9				8	16619.728	0.002	16619.693	0.004
1	9	8	8	0	8	7	16821.568	0.003	16821.800	0.004
		10				9	16821.591	0.000	16821.824	0.000
		9				8	16821.634	0.001	16821.867	0.002
1	10	9	9	1	9	8	18480.820	0.002	18481.022	0.002
		11				10	18480.834	0.000	18481.038	0.002
		10				9	18480.834	0.002	18481.038	0.000
0	10	9	9	0	9	8	18516.726	0.003	18516.428	0.002
		11				10	18516.740	0.002	18516.441	0.002
		10				9	18516.753	0.001	18516.456	0.004
	1 2 3 2 1 1 0	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$egin{array}{cccccccccccccccccccccccccccccccccccc$	$egin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	1 7 7 7 1 6 6 16196.483 0.001 9 - - 8 16196.518 0.004 2 4 5 5 1 4 4 16234.658 0.003 2 4 5 5 1 4 4 16234.658 0.003 2 4 5 5 1 4 4 16234.658 0.003 3 5 8 7 3 4 7 16256.327 0.002 3 9 - - 6 16256.548 0.000 4 16619.711 0.001 6 16619.711 0.001 7 7 2 5 7 16619.643 0.002 1 9 8 8 0 8 16619.711 0.001 10 10 - - 8 16619.713 0.001 11 - - 8 16821.634 0.002 10 9 9 1	1 7 7 1 6 6 16196.483 0.001 16196.400 9 8 8 16196.518 0.004 16196.430 8 7 16196.557 0.004 16196.433 2 4 5 5 1 4 4 16234.658 0.003 16234.389 2 4 5 5 1 4 4 16234.658 0.003 16234.475 6 16234.743 0.002 16234.475 0.002 16234.658 3 5 8 7 3 4 7 16256.327 0.002 16256.042 9 7 2 5 7 16619.643 0.001 16619.615 10 7 2 5 7 16619.643 0.001 16619.615 10 7 2 5 7 16619.643 0.001 16619.615 10 8 16256.548 0.002 16619.615 16619.615 16619.615 10 9 8 16619.728 <td< td=""></td<>

CHAPTER 5

MICROWAVE SPECTROSCOPY STUDY OF PROLINE METHYL ESTER: RING PUCKERING AND BARRIER TO METHYL INTERNAL ROTATION

5.1 Introduction

The structures of small gas-phase biological molecules can be determined precisely by high-resolution rotational spectroscopy. This technique has been widely used to investigate different varieties of molecules in their neutral form isolated in the gas phase ^{9,62,63}. The conformational behavior of amino acids and their derivatives is important to better understand the role of structure in polypeptide and protein function. Rotational spectroscopic studies of several amino acids and their derivatives have been reported previously. Conformational analysis of the amino acid glycine – the smallest amino acid – found two energetically different structures ^{3–8}. The higher energy conformer, found first because of its larger dipole moment, has an intramolecular hydrogen bond from the carboxylic acid to the amino nitrogen³. The lowest energy conformation – observed later – has bifurcated intramolecular hydrogen bonds from the amine group to the carbonyl oxygen⁷.

Laser-ablation Fourier-transform microwave spectroscopy has been employed for many neutral amino acid investigations including, proline⁶², and valine⁹. Rotational spectra of the two lowest energy conformers of proline were reported first ⁶². Unlike for glycine, the lowest energy conformers of proline have a *trans*-COOH arrangement with an intramolecular hydrogen bond to the imino nitrogen. The conformers are distinguished by different ring puckering: C_{γ}-endo (lowest energy) and C_{γ}-exo (+3.2 kJ mol⁻¹). Proline conformers with the cis-COOH arrangement and an intramolecular hydrogen bond from the imino hydrogen to the carbonyl oxygen were calculated to be higher in energy (8.9 kJ mol⁻¹ and 9.4 kJ mol⁻¹); spectra from these conformers were found and reported later⁶³. The higher-energy conformers have C_{γ} -exo and C_{γ} -endo ring puckering, respectively. The microwave spectrum of neutral value was used to identify the structures of two different conformations, with the same orientation of the isopropyl group ($\chi_1 = H^{\beta} - C^{\beta} - C^{\alpha} - H^{\alpha}$ $\approx 300^{\circ}$) for each of the two backbone conformations observed for glycine.

The amino acid derivatives prolinamide and valinamide are stabilized by intramolecular hydrogen bonds from the amide to the amino nitrogen. Only one conformation of valinamide, with the isopropyl side group oriented with $\chi_1 = 298^\circ$, was identified from its rotational spectrum ⁴². The rotational spectrum of prolinamide, also arises from a single conformation with an intramolecular hydrogen bond from amide to the imino nitrogen⁴¹.

We reported the rotational spectra of two conformers of valine methyl ester in chapter 4 ⁶⁴. Both conformers are stabilized by a network of bifurcated hydrogen bonds from the amine to the carbonyl oxygen, similar to the lowest energy conformation of glycine, with two different orientations of the isopropyl side chain: $\chi_1 = -64.0^\circ$ and $\chi_1 = 173.6^\circ$. Interestingly, the conformer with $\chi_1 = 173.6^\circ$ had not been previously detected in the rotational spectra of neutral valine or valinamide. Derivatization of the amino acid backbone structure affects the intramolecular hydrogen bonding networks and may also influence the relative energies for side chain orientations and conformational preferences.

The rotational spectra of amino acid methyl esters display tunneling splittings arising from methyl internal rotation. Methyl rotation is restricted by a three-fold potential which depends on the local electronic environment of the methyl group¹⁰. Tunneling results in symmetric (A) and

antisymmetric (*E*) states which are populated by different, noninterchangeable sets of ¹H nuclear spins, and which give rise to separate rotational transitions that appear as splittings. Assignment of the *A* and *E* tunneling components in a rotational spectrum can be used to find the potential-energy barrier and the orientation of the methyl rotor.

Methyl internal rotation splittings have been observed for numerous biomimetic species including *N*-acetyl-alanine-*N*-methylamide ⁴⁵, ethylacetamidoacetate ⁴⁶, *N*-acetylglycine ⁴⁷, a series of methyl indoles ¹⁰ and *N*-acetyl alanine methyl ester ⁴⁸. Three amino acid methyl ester related studies have been reported previously: glycine methyl ester ⁴⁹, *N*-acetyl alanine methyl ester ⁴⁸, and valine methyl ester ⁶⁴. The rotational spectrum of glycine methyl ester was assigned to a single conformer⁴⁹, and the methyl *V*₃ barrier was found to be 411.66(10) cm⁻¹. The two different methyl groups in *N*-acetyl alanine methyl ester cause a complicated pattern of tunneling pairs (*AA*, *AE*, and *EA*); the barriers for the methoxy and acetyl methyl groups were found to be 396.46(7) cm⁻¹ and 64.96(4) cm⁻¹, respectively. The reported *V*₃ barriers for valine methyl ester were found to be 401.64 cm⁻¹, for conformer I and 409.74 cm⁻¹ for conformer II.

In this investigation we report the rotational spectra and conformational structures of proline methyl ester (PrOMe), compared to neutral proline and prolinamide in Figure 1. Ester formation blocks acid-to-imino nitrogen hydrogen bonding, and the puckering of the pyrrolidine ring side chain may also be influenced by ester derivatization.



Figure 5.1. Numbering scheme for (A) PrOMe and comparison to the structures of (B) neutral proline and (C) prolinamide.

5.2 Experimental and Computational Methods

L-Proline methyl ester hydrochloride (98% pure) was purchased from Sigma-Aldich. Free L-proline methyl ester was isolated following a previously reported procedure⁶⁵. In an oven-dried 100 mL rb flask, L-proline methyl ester hydrogen chloride salt (10. 0 g, 60.6 mmol, 1 eq) was mixed with chloroform (45 mL) under argon gas. A solution of triethylamine (8.44 mL, 60. 6 mmol, 1 eq) in chloroform was added dropwise. The reaction mixture was stirred for 4 hours at room temperature and then heated to reflux for an hour. The reaction mixture was cooled to room temperature and concentrated to obtain a white solid. The obtained crude was diluted with diethyl ether, filtered, and washed with diethyl ether. The filtrate was concentrated to obtain L-proline methyl ester as a free amino ester (7.05 g, 90%, yellow oil). ¹H NMR (400 MHz, CDCl₃) δ 3.74 (dd, *J* = 8.7, 5.7 Hz, 1H), 3.69 (s, 3H), 3.04 (dt, *J* = 10.2, 6.7 Hz, 1H), 2.93 – 2.82 (m, 1H), 2.45 (s, 1H), 2.17 – 2.03 (m, 1H), 1.86 – 1.68 (m, 3H). Characterization data are consistent with the reported literature⁶⁶.

A high-resolution mini cavity-based Fourier-transform microwave (FTMW) spectrometer was used to record the rotational spectrum of proline methyl ester in the 9-18 GHz frequency range. The detailed information of the instrument has been described in elsewhere³¹. Approximately 0.5 mL of free PrOMe was placed in the reservoir nozzle³⁵, heated to 85 °C using a Watlow band heater and an Omega CN8201 temperature controller, and carried into the Fabry-Perot resonant cavity with Argon gas at 1 atm backing pressure. The coaxial expansion into the resonator cavity results in a ca. 60 kHz Doppler splitting of the observed transitions. The frequency resolution of the spectrometer is 2.5 kHz; two resolved portions of the microwave spectrum of PrOMe are shown in Figure 5.2 and 5.3. GAUSSIAN 16^{37} on the Owens cluster at the Ohio Supercomputer Center was used to perform high level DFT and *ab initio* calculations. The initial input structures were created using Avogadro – an advanced molecule editor – by adjusting the orientations of the methoxy and carbonyl groups as well as the structure of the pyrrolidine ring. Full geometry optimizations of fifteen different starting structures of PrOMe were carried out using four levels of theory – ω B97XD/6-311++G(d,p), MP2/6-311++G(d,p), and ω B97XD/aug-cc-pVDZ and MP2/aug-ccpVDZ. Initial fitting of the spectrum was carried out using the RRFIT, ZFAP, and QUAD2I programs using Watson *A*-reducion Hamiltonian. Final spectral fitting to rotational constants, centrifugal distortion constans, and nuclear quadrupole coupling constants, and including both *A* and *E* methyl internal rotation tunneling states, was done using XIAM⁵⁵.

5.3 Results

Four levels of theory – ω B97XD/6-311++G(d,p), MP2/6-311++G(d,p), and ω B97XD/aug-cc-pVDZ and MP2/aug-cc-pVDZ – were employed to optimize the 15 different staring conformers of PrOMe. Nine different conformational minima were identified in DFT and MP2 calculations. The conformational minima under 10 kJ mol⁻¹ of the relative energy for both DFT and MP2 calculations were considered for further calculations (shown in figure 5.5, 5.6, 5.7, and 5.8). Tables 5.1, 5.2, 5.3, and 5.4 provide the relative energies, theoretically calculated rotational constants, dipole moment projections, ΔI_{rms} values, second moments, and information about the ring puckering and the hydrogen bonds (see figure 5.4 for H bond identification).

A rich spectrum with 51 rotational transitions – 16 *a*-type, 18 *b*-type, and 5 *c*-type – was assigned for PrOMe including 39 R-branch and 12 Q-branch transitions. Each rotational transition consists of hyperfine components which were able to assign and fit using XIAM⁵⁵. 293 ¹⁴N nuclear

hyperfine components - 144 of *A*-state components and 149 of *E*-state components - were fit to 51 rotational transition center frequencies and the nuclear quadrupole coupling constants: $\chi_{aa} = 2.302(6) \chi_{bb} - \chi_{cc} = 5.487(9)$ MHz for the lowest energy conformer of PrOMe. Several hyperfine components could not be resolved as they overlap each other. The frequencies of all resolved hyperfine components for PrOMe are available in table 5.7.

Final fitting of the assigned transitions of PrOMe was carried out using XIAM and the A-reduction Hamiltonian. The assigned spectrum was fit to rotational constants, nuclear quadrupole cupling constants, quartic centrifugal distortion constants, and the barrier to methyl internal rotation (V_3) . Table 5.5 and 5.6 compare these experimentally determined rotational spectroscopic parameters with the theoretical model parameters of conformers I and III from the four levels of theory.



Figure 5.2. Portion of the microwave spectrum of PrOMe showing (*A*) and (*E*) tunneling state components and nuclear quadrupole hyperfine components of the 5_{15} - 4_{04} rotational transition.



Figure 5.3. Portion of the microwave spectrum of PrOMe showing (*A*) and (*E*) tunneling state components and nuclear quadrupole hyperfine components of the 7_{07} - 6_{16} rotational transition.



Figure 5.4. Identification of intramolecular hydrogen bonds in model structures. (A) $NH \cdots O_{carbonyl}$ (H-acceptor is carbonyl oxygen) and (B) $NH \cdots O_{ester}$ (H-acceptor is ester oxygen).





Conformer I





Conformer III



Conformer IV



Conformer V

Table 5.1. Theoetic	cally calculated spectro	oscopic parameters	for the lowest	energy PrOMe
structures at the ωE	397XD/6-311++G(d,p) level of theory.		

Parameter	Conformer I	Conformer II	Conformer III	Conformer IV	Conformer V
Pucker	C^{δ} endo	C^{β} exo	C^{γ} endo	C^{δ} endo	C^{γ} exo
$\Delta E / \text{kJ mol}^{-1}$	0.00	5.85	7.14	9.20	9.25
A/MHz	3758.486	2843.710	3694.060	3946.938	3267.807
<i>B</i> /MHz	1030.287	1245.824	1036.091	976.001	1048.230
C/MHz	895.685	1070.704	920.222	927.096	969.866
$\mu_a/{ m D}$	-0.77	0.34	0.44	0.68	-0.77
$\mu_b/{ m D}$	1.86	1.91	-1.64	0.83	1.69
$\mu_c/{ m D}$	-0.58	-0.83	1.41	-1.84	-2.07
ΔI_{rms} / amu Å ²	16.9	63.9	8.1	19.4	13.2
P_{aa} / amu Å ²	460.149	349.973	449.889	467.442	424.277
P_{bb} / amu Å ²	104.089	122.033	99.304	77.680	96.805
P_{cc} / amu Å ²	30.375	55.685	37.505	50.364	57.850
H-acceptor	Carbonyl	Ester	Ester	-	Ester



Conformer I



Conformer III



Conformer V



Conformer II



Conformer IV



Conformer VI

Figure 5.6. Optimized structures of the model conformers of PrOMe at the MP2/6-311++G(d,p) level of theory.

Parameter	Conformer I	Conformer II	Conformer III	Conformer IV	Conformer V	Conformer VI
Pucker	C^{δ} endo	C ^β exo	C^{γ} endo	C^{δ} endo	C^{γ} exo	C^{γ} endo
$\Delta E / \text{kJ mol}^{-1}$	0.00	5.10	6.23	7.38	7.54	9.21
A/MHz	3685.050	2781.553	3682.924	3911.323	3286.511	3001.758
<i>B</i> /MHz	1042.925	1279.560	1038.205	980.351	1034.659	1151.208
C/MHz	912.497	1102.136	929.108	945.156	973.138	1031.830
μ_a/D	-0.93	0.53	0.44	0.64	-0.72	-1.66
$\mu_b/{ m D}$	1.57	1.79	-1.42	0.47	1.61	-1.31
$\mu_c/{ m D}$	-0.69	-0.89	1.36	-1.67	-2.05	0.39
ΔI_{rms} / amu Å ²	10.8	73.8	5.0	17.1	13.2	42.2
P_{aa} / amu Å ²	450.639	335.910	446.750	460.502	427.003	380.213
P_{bb} / amu Å ²	103.204	122.636	97.189	74.203	92.327	109.576
P_{cc} / amu Å ²	33.940	59.054	40.031	55.006	61.447	58.785
H-acceptor	Carbonyl	Ester	Ester	-	Ester	Carbonyl

Table 5.2. Theoretically calculated spectroscopic parameters for the lowest energy PrOMe structures at the MP2/6-311++G(d,p) level of theory.





Conformer I

Conformer II



Conformer III



Conformer IV

Figure 5.7. Optimized structures of the model conformers of PrOMe at the ω B97XD/aug-ccpVDZ level of theory.

Table 5.3. Theoretically calculated spectroscopic parameters for the lowest energy PrOMe structures at the ω B97XD/aug-cc-pVDZ level of theory.

Parameter	Conformer I	Conformer II	Conformer III	Conformer IV
Pucker	C^{δ} endo	N exo	C^{γ} endo	C^{δ} endo
$\Delta E / \text{kJ mol}^{-1}$	0.0	6.26	7.99	9.77
A/MHz	3728.494	3114.908	3693.704	3906.991
<i>B</i> /MHz	1031.147	1168.661	1030.297	979.189
C/MHz	895.728	994.270	907.991	933.001
μ_a/D	-0.70	-066	0.46	0.67
μ_b/D	1.87	-1.70	-1.71	0.67
μ_c/D	-0.51	-0.34	1.40	-1.88
ΔI_{rms} / amu Å ²	16.9	38.0	12.5	17.8
P_{aa} / amu Å ²	459.389	389.245	455.143	464.219
P_{bb} / amu Å ²	104.820	119.047	101.447	77.451
P_{cc} / amu Å ²	30.724	43.198	35.375	51.901
H-acceptor	Carbonyl	Ester	Ester	-



Conformer I



Conformer II



Conformer III



Conformer V



Conformer IV



Conformer VI

Parameter	Conformer I	onformer Conformer Conform II III		Conformer IV	Conformer V	Conformer VI
Pucker	C^{δ} endo	C^{β} exo	C^{γ} endo	C^{δ} exo	C^{δ} endo	C^{γ} endo
$\Delta E / \text{kJ mol}^{-1}$	0.0	5.22	7.30	7.57	7.65	9.98
A/MHz	3580.709	2733.508	3650.571	3214.826	3836.143	3000.078
<i>B</i> /MHz	1048.535	1280.287	1029.297	1038.289	981.053	1131.498
C/MHz	918.790	1104.861	920.415	975.513	951.919	1017.232
μ_a/D	-0.76	-0.12	0.66	-1.09	0.81	-2.06
μ_b/D	-1.82	-2.12	-1.69	-1.92	0.30	-1.52
μ_c/D	0.46	-0.68	1.65	2.04	-2.01	0.38
∆I _{rms} ⁄ amu Å ²	9.3	74.9	8.3	15.1	16.7	36.9
P_{aa} / amu Å ²	445.447	333.635	450.816	423.802	457.151	387.504
P_{bb} / amu Å ²	104.600	123.779	98.261	94.263	73.750	109.313
P_{cc} / amu Å ²	36.538	61.104	40.178	62.940	57.98	59.142

Figure 5.8. Optimized structures of the model conformers of PrOMe at the MP2/aug-ccpVDZ level of theory.

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H-acceptor	Carbonyl	Ester	Ester	Ester	-
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Table 5.5. Comparison between experimentally determined rotational spectroscopicparameters of PrOMe with the model parameters of conformer I calculated from four differentlevels of theory.

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		Theoretical	Theoretical	Theoretical	Theoretical
Parameter	Experimental	(ωB97XD/6-	(MP2/6-	(@B97XD/aug-	(MP2/aug-
	(XIAM)	311++G(d,p))	311++G(d,p))	cc-pVDZ)	cc-pVDZ)
A/MHz	3678.4360(7)	3758.486	3685.050	3728.494	3580.708
<i>B</i> /MHz	1037.5616(3)	1030.287	1042.925	1031.147	1048.535
C/MHz	944.2045(3)	895.685	912.497	895.728	918.790
⊿ _J /kHz	0.423(15)				
<i>∆_{JK}</i> /kHz	-2.889(7)				
Δ_k/kHz	11.41(6)				
$\delta_J/{ m kHz}$	0.0367(8)				
$\delta_{\it K}$ /kHz	0.97(9)				
χ_{aa} / MHz	2.302(6)	1.002	0.440	0.821	0.022
χ_{bb} - χ_{cc} / MHz	5.487(9)	5.969	4.933	5.481	3.895
V_3 / cm ⁻¹	393.54(9)				
P_{aa} / amu Å ²	442.468	460.149	450.639	459.389	445.447
P_{bb} / amu Å ²	92.775	104.089	103.204	104.820	104.600
P_{cc} / amu Å ²	44.615	30.375	33.940	30.724	36.538

$\angle(i, a)$ /°	28.4(11)	21.5	23.4	24.4	28.02
$\angle(i, b)$ /°	76.4(5)	78.4	76.3	74.9	76.01
$\angle(i, c)$ /°	114.4(4)	114.4	116.4	113.2	113.9
N^a	51				
No. $A \operatorname{lines}^b$	144				
No. $E \text{ lines}^b$	149				
RMS/kHz	7.4				

Table 5.6. Comparison between experimentally determined rotational spectroscopic parameters of PrOMe with the model parameters of conformer III calculated from four different levels of theory.

	Even a sine a setal	Theoretical	Theoretical	Theoretical	Theoretical
Parameter		(ωB97XD/6-	(MP2/6-	(@B97XD/aug-	(MP2/aug-
	(AIAM)	311++G(d,p))	311++G(d,p)) 311++G(d,p))		cc-pVDZ)
A/MHz	3678.4360(7)	3694.060	3682.924	3693.704	3650.571
<i>B</i> /MHz	1037.5616(3)	1036.091	1038.205	1030.297	1029.297
C/MHz	944.2045(3)	920.222	929.108	907.991	920.415
⊿ _J /kHz	0.423(15)				
Δ_{JK}/kHz	-2.889(7)				
Δ_k/kHz	11.41(6)				
δ_{J}/kHz	0.0367(8)				
$\delta_{\it K}$ /kHz	0.97(9)				
χ_{aa} / MHz	2.302(6)	0.981	0.890	1.030	0.775
χ_{bb} - χ_{cc} / MHz	5.487(9)	6.272	5.599	6.060	4.983
$V_3 / { m cm}^{-1}$	393.54(9)				

P_{aa} / amu Å ²	442.468	449.889	446.750	455.143	450.816
P_{bb} / amu Å ²	92.775	99.304	97.189	101.447	98.261
P_{cc} / amu Å ²	44.615	37.505	40.031	35.375	40.178
$\angle(i, a) /^{\circ}$	28.4(11)	25.8	26.8	26.9	30.4
$\angle(i, b)$ /°	76.4(5)	80.6	79.7	76.2	77.4
$\angle(i, c)$ /°	114.4(4)	118.3	120.1	117.2	117.5
N^a	51				
No. A lines ^{b}	144				
No. $E \operatorname{lines}^{b}$	149				
RMS/kHz	7.4				

5.4 Discussion

The amino acid derivative PrOMe was chosen for this experiment to continue the conformational analysis of the amino acid methyl ester series because the ring-puckering and the H-bonding network were interesting to study in this molecule. The previous investigations neutral proline^{62,63} and prolinamide⁴¹ also provided detailed information on ring-puckering and H-bonding of their molecular systems.

For PrOMe, four low-energy structures from each level of theory were considered for further discussion. The *ab initio* calculations from all four levels of theory, ω B97XD/6-311++G(d,p), MP2/6-311++G(d,p), and ω B97XD/aug-cc-pVDZ and MP2/aug-cc-pVDZ, produce the same lowest energy model structure, conformer I that has an intramolecular H bond from imino hydrogen to carbonyl oxygen (N-H...O=C) and C^{δ} endo puckering in the ring. The model conformers II and III have the same network of intramolecular hydrogen bonds involving the imino hydrogen to ester oxygen (N-H...O–C) and the ring-puckering, C^{β} exo and C^{γ} endo for conformer II, III respectively, except the unique N exo puckering of the conformer II from ω B97XD/aug-cc-pVTZ level of theory. For conformer IV, only the predicted model from MP2/aug-cc-pVDZ level of theory shows an intramolecular H bond between imino hydrogen and ester oxygen and shows unique C⁶ exo puckering from MP2/aug-cc-pVTZ level of theory and C^{γ} endo puckering for all other three levels of theory. The ring-puckering of neutral proline^{62,63} shows C^{γ} endo and C^{γ} exo like structures while the microwave spectroscopic study of prolinamide confirms a twist C^{β} exo/C^{γ} endo conformation⁴¹.

The intramolecular H-bond scheme of PrOMe is different from the H-bond networking of neutral proline^{62,63}, and prolinamide⁴¹. Neutral proline has two different intramolecular H-bond networks – from hydroxyl group to imine nitrogen (O-H...N) and from imine hydrogen to carbonyl oxygen (N-H...O=C) 62,63 – while prolinamide consists of an intramolecular H-bond from amide hydrogen and imine nitrogen (N-H...N) 41 .

Two stable conformers of PrOMe were identified in a recent computational and spectroscopic study at the ω B97XD/aug-cc-pVTZ and MP2/aug-cc-pVTZ levels of theory⁵². This ¹H NMR and IR spectroscopic study also showed the population of the lowest energy conformer is about 90%, both in isolated (gas) and solution phases⁶⁷. Calculated energies demonstrated that the lowest energy conformer is 5.4 kJ mol⁻¹ more stable than the other stable conformer. The lowest energy conformer has a cis arrangement with the N – C – C = O dihedral angle ($\approx 6^{\circ}$) which facilitates a likely H-bond from imino hydrogen to carbonyl oxygen (N-H...O=C); whereas in second conformer shows a trans arrangement of the dihedral angle, N – C – C = O $\approx 155^{\circ}$ and a possible H-bond between imine hydrogen and methoxy oxygen (N-H...O–C)⁶⁷.

The ΔI_{rms} values and second moments can be used to identify the structure of a molecule because the moment of inertia and the second moment are directly related to the structure. The ΔI_{rms} values and second moments for model conformers are given in tables 5.1, 5.2, 5.3, and 5.4 and table 5.5 and 5.6 compare the experimentally determined second moments with the second moments calculated for conformers I and III respectively. The ΔI_{rms} values and second moments are rather close for conformers I and III, and assignment of the experimental structure remains uncertain. Therefore additional factors should also be considered.

The dipole moments of the model structures can be considered as a key parameter for assigning the experimental structure. In the experimental spectrum, the intensities of the three types of transitions reflect the dipole moment projections of the experimental structure. By comparing at the dipole moment data of PrOMe provided in tables 5.1-5.4 and the experimental spectrum, we can identify the structure most likely associated with the experimental spectrum. The dipole moment projections of conformer I predict the rotational spectrum best: higher intensities for b-type transitions and lower intensities for c-type transitions ($\mu_b = 1.57 \text{ D} > \mu_c = -0.69 \text{ D}$). The models for conformer III predict $\mu_b = -1.42 \text{ D} \approx \mu_c = 1.36 \text{ D} > \mu_a = 0.44 \text{ D}$, with nearly identical intensities for b- and c- type transitions. Figure 5.9 shows a comparison of b-type transition took 13081 shots to show resolvable splittings, confirming the experimental spectrum is in better agreement with the predicted dipole moment of conformer I.

The rotor-axis angles from fitting tunneling splittings can also be used as an important parameter of determining the experimental structure of a molecule. Table 5.5 and 5.6 show the comparison of experimental rotor-axis angles with predicted rotor-axis angles from all four levels of theory for conformers I and III respectively. The rotor-axis angles of conformer I from MP2/aug-cc-pVDZ level of theory are well matched with the experimental rotor-axis angles. Overall the predicted angles of conformer I from all four levels of theory are in closer agreement with the experimental values compared to the predicted angles of conformer III. Rotor-axis angles confirm the assignment of the experimental structure of PrOMe to conformer I.

In the microwave spectrum of PrOMe recorded from cavity-based FTMW spectrometer, 51 rotational transitions were fitted to their *A* and *E* tunneling states. The barrier to methyl internal rotation was calculated to be 393.54(9) cm⁻¹. The experimental values of the V_3 barrier of PrOMe was also within the range of values from previously investigated amino acid methyl esters: glycine methyl ester (411.66(10) cm⁻¹)⁴⁹, valine methyl ester(conformer I: 401.64(19) cm⁻¹, conformer II: 409.74(16) cm⁻¹)⁶⁴, and N-acetyl alanine methyl ester (396.46(7) cm⁻¹)⁴⁸.



Figure 5.9. Portions of the microwave spectrum of PrOMe showing a b-type transition (120 shot averaged) and a c-type transition (13000 shot averaged).

5.5 Conclusion

The pure rotational spectrum of PrOMe was recorded in 9-18 GHz frequency range by high resolution FTMW spectrometer for the first time. Full geometry optimizations were carried out using four levels of theory – ω B97XD/6-311++G(d,p), MP2/6-311++G(d,p), and ω B97XD/aug-cc-pVDZ and MP2/aug-cc-pVDZ – which were used to provide the guidance for spectral assignment and detailed molecular structure information. All four different levels of theory predicted the same conformation – conformer with C⁸ endo ring-puckering and intramolecular H-bond from imine to carbonyl oxygen ((N-H...O=C) – as the lowest energy structure of PrOMe. But assigning the experimental structure to a model structure was not an easy task, because the predicted third lowest energy structure from all the above four levels of calculations were also in good agreement with the experimental moments of inertia. After consideration of the calculated rotational constants, dipole moments, second moments of inertia, and the rotor-axis angles, we concluded the predicted lowest energy structure of PrOMe has better agreement with the experimental structure.

5.6 Optimized Molecular Coordinates for PrOMe conformer

Table 5.7. Optimized Molecular Coordinates	(Å) for PrOMe conformer
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Atom Number	Atom	a	b	с
1	Н	3.158947	1.090325	0.319197
2	Н	1.039717	2.272220	0.512193
3	С	2.425737	0.833997	-0.460853
4	С	1.020357	1.340905	-0.070598
5	Н	2.769037	1.244224	-1.422292
6	Н	0.416580	1.527572	-0.972566
7	Н	3.165351	-1.252123	-0.435221
8	С	2.225206	-0.683138	-0.489746
9	Н	0.188576	0.471011	1.786692
10	С	0.406282	0.160127	0.751648

11	ч	1 681283	-0 978666	-1 409955
1 1 1	11	1.001205	0.970000	1.409900
12	0	-1.105886	-1.365153	-0.418820
13	Ν	1.429656	-0.896369	0.737000
14	С	-0.910035	-0.288903	0.130602
15	Н	0.973646	-1.810995	0.715571
16	0	-1.859192	0.674054	0.263107
17	н	-3.026218	0.146312	-1.400034
18	С	-3.139089	0.325229	-0.321875
19	н	-3.540479	-0.578032	0.157601
20	н	-3.785145	1.189550	-0.133127

5.7 Assigned transition frequencies for PrOMe conformer.

 Table 5.8. Assigned transition frequencies for PrOMe conformer.

J'	K'_a	K_c'	F'	J‴	$K_a^{\prime\prime}$	$K_c^{\prime\prime}$	$F^{\prime\prime}$	A obs	A obs-calc	E obs	E obs-calc
								(MHz)	(MHz)	(MHz)	(MHz)
5	1	5	6	4	1	4	5			9665.038	0.002
			4				3			9665.111	0.005
			5				4			9665.129	0.009
6	0	6	6	5	1	5	5	9750.784	0.008	9751.057	0.008
			7				6	9751.366	0.010	9751.638	0.005
			5				4	9751.523	0.011	9751.797	0.006
5	0	5	6	4	0	4	5	9860.480	0.004	9860.444	0.002
			4				3	9860.515	0.000	9860.480	0.004
			5				4	9860.580	0.002	9860.547	0.008
5	2	4	4	4	2	3	3	9903.276	0.009	9905.910	0.002
			6				5	9903.297	0.006	9905.934	0.004
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			5				4	9903.471	0.009	9906.118	0.002
5	1	4	4	4	1	3	3	10130.907	0.020	10130.877	0.008
			6				5	10130.958	0.006	10130.937	0.028
			5				4	10131.014	0.005	10130.977	0.010
4	1	4	3	3	0	3	2	10153.582	0.002	10153.228	0.004
			5				4	10153.708	0.002	10153.351	0.004
			4				3	10154.489	0.002	10154.130	0.002
4	1	3	4	3	0	3	3	11086.280	0.010	11086.068	0.004
								11087.370	0.007	11087.156	0.004
								11087.722	0.014	11087.513	0.003
6	1	6	7	5	1	5	6	11591.883	0.007	11591.861	0.001
			5				4	11591.932	0.006	11591.906	0.003
			6				5	11591.955	0.002	11591.932	0.008
6	0	6	7	5	0	5	6	11806.915	0.006	11806.871	0.000
			5				4	11806.938	0.001	11806.892	0.003
			6				5	11807.021	0.005	11806.983	0.008
6	2	5	5	5	2	4	4	11879.423	0.007	11880.575	0.007
			7				6	11879.423	0.009	11880.575	0.004
			6				5	11879.537	0.008	11880.692	0.004
7	0	7	7	6	1	6	6	11899.724	0.010	11899.963	0.008
			8				7	11900.256	0.012	11900.497	0.007
			6				5	11900.376	0.013	11900.618	0.008
5	1	5	4	4	0	4	3	11915.919	0.001	11915.578	0.004
			6				5	11916.031	0.002	11915.688	0.005
			5				4	11916.812	0.001	11916.469	0.004
2	2	1	1	1	1	0	0	11978.570	0.012	11963.879	0.002
			2				2	11979.180	0.003		
			3				2	11979.991	0.013	11965.202	0.006
			2				1	11980.340	0.012	11965.605	0.004
2	2	1	1	1	1	1	1	12073.480	0.001	12059.885	0.005

			3				2	12073.540	0.005	12059.946	0.007
2	2	0	1	1	1	1	1	12075.930	0.003	12088.984	0.006
			3				2	12075.980	0.003	12089.040	0.003
6	1	5	5	5	1	4	4	12149.701	0.009	12149.668	0.001
			7				6	12149.716	0.008	12149.687	0.004
			6				5	12149.767	0.005	12149.738	0.007
9	3	6	8	9	2	7	8	12931.872	0.001	12940.258	0.000
			10				10	12931.906	0.002	12940.293	0.002
			9				9	12932.189	0.000	12940.584	0.000
8	3	6	7	8	2	6	7	13084.128	0.011	13067.914	0.005
			9				9	13084.180	0.014	13067.943	0.003
			8				8	13084.386	0.000	13068.150	0.006
8	3	5	7	8	2	6	7	13102.288	0.001	13114.844	0.001
			9				9	13102.316	0.003	13114.871	0.003
			8				8	13102.500	0.002	13115.067	0.000
7	3	4	6	7	2	5	6	13229.658	0.002	13245.489	0.001
			8				8	13229.672	0.001	13245.503	0.001
			7				7	13229.749	0.003	13245.586	0.003
6	3	3	6	6	2	4	6	13318.756	0.008	13336.244	0.005
				7			7	13318.832	0.006	13336.308	0.008
5	3	2	5	5	2	3	5	13376.324	0.001	13393.691	0.002
			6				6	13376.554	0.004	13393.926	0.002
			4				4	13376.606	0.001	13393.978	0.001
4	3	1	4	4	2	2	4	13409.935	0.002	13425.060	0.000
			5				5	13410.415	0.001	13425.560	0.007
			3				3	13410.533	0.005	13425.673	0.007
4	3	2	4	4	2	3	4	13445.902	0.003	13428.033	0.000
			5				5	13446.528	0.002	13428.641	0.002
			3				3	13446.681	0.004	13428.793	0.002
5	3	3	5	5	2	4	5	13459.519	0.001	13439.404	0.003
			6				6	13459.973	0.002	13439.852	0.000

			4				4	13460.065	0.003	13439.960	0.016
6	3	4	6	6	2	5	6	13482.961	0.003	13462.729	0.002
			7				7	13483.334	0.004	13463.102	0.004
			5				5	13483.404	0.004	13463.177	0.007
6	3	3	6	6	2	5	6			13506.228	0.001
			7				7			13506.603	0.001
			5				5			13506.673	0.006
7	1	7	8	6	1	6	7	13515.687	0.001	13515.650	0.003
			6				5	13515.717	0.005	13515.679	0.003
			7				6	13515.737	0.004	13515.706	0.005
7	3	5	7	7	2	6	7	13519.844	0.003	13501.244	0.002
			8				8	13520.179	0.003	13501.583	0.003
			6				6	13520.231	0.002	13501.637	0.001
6	1	6	5	5	0	5	4	13647.342	0.001	13647.009	0.003
			7				6	13647.435	0.000	13647.103	0.005
			6				5	13648.186	0.001	13647.853	0.003
7	0	7	8	6	0	6	7	13740.774	0.007	13740.720	0.003
			6				5	13740.788	0.005	13740.733	0.005
			7				6			13740.831	0.001
7	2	6	8	6	2	5	7	13853.068	0.008	13853.581	0.006
			6				5	13853.068	0.011	13853.581	0.003
			7				6	13853.154	0.004	13853.666	0.008
3	2	2	2	2	1	1	1	13867.991	0.013	13857.353	0.006
			4				3	13868.273	0.016	13857.628	0.006
			3				2	13868.785	0.016	13858.112	0.003
3	2	1	2	2	1	1	1	13880.181	0.013	13888.791	0.003
			4				3	13880.436	0.015	13889.057	0.004
			3				2	13880.870	0.014	13889.511	0.002
7	3	5	6	6	3	4	5	13889.900	0.008	13892.048	0.004
			8				7	13889.914	0.006	13892.061	0.006
			7				6	13890.031	0.010	13892.178	0.005

7	3	4	6	6	3	3	5	13894.858	0.009	13892.666	0.006
			8				7	13894.871	0.007	13892.679	0.008
			7				6	13894.982	0.008	13892.794	0.008
7	2	5	7	6	2	4	6	13983.996	0.012	13983.448	0.005
			8				7	13984.023	0.010	13983.476	0.007
			6				5	13984.034	0.009	13983.488	0.008
8	0	8	8	7	1	7	7	14045.768	0.012	14045.971	0.011
			9				8	14046.237	0.012	14046.439	0.010
			7				6	14046.329	0.013	14046.532	0.010
3	2	2	3	2	1	2	2	14147.463	0.007	14137.171	0.008
			4				3	14148.718	0.003	14138.442	0.005
			2				1	14149.407	0.010	14139.147	0.006
3	2	1	3	2	1	2	2	14159.563	0.009	14168.568	0.005
			4				3	14160.889	0.005	14169.871	0.003
			2				1	14161.612	0.005	14170.584	0.002
7	1	6	6	6	1	5	5	14164.079	0.010	14164.020	0.005
			8				7	14164.095	0.016	14164.034	0.001
			7				6	14164.142	0.017	14164.079	0.003
7	1	7	6	6	0	6	5	15356.123	0.003	15355.809	0.009
			8				7	15356.201	0.001	15355.871	0.003
			7				6	15356.901	0.001	15356.578	0.002
4	2	3	3	3	1	2	2	15709.435	0.016	15703.462	0.010
			5				4	15709.631	0.017	15703.653	0.008
			4				3	15710.281	0.018	15704.284	0.004
4	2	2	3	3	1	2	2	15745.876	0.001	15.749.993	0.010
			5				4	15746.033	0.003	15750.153	0.006
			4				3	15746.539	0.004	15750.675	0.005
8	2	6	8	7	2	5	7	16015.226	0.012	16014.947	0.005
			9				8	16015.276	0.010	16015.000	0.009
			7				6	16015.289	0.008	16015.011	0.009
9	0	9	9	8	1	8	8	16179.839	0.011	16179.993	0.014

			10				9	16180.234	0.013	16180.384	0.009
			8				7	16180.320	0.002	16180.463	0.016
4	2	2	4	3	1	3	3	16305.231	0.023	16309.542	0.005
			5				4	16306.550	0.020	16310.847	0.004
			3				2	16307.028	0.019	16311.322	0.004
8	1	8	7	7	0	7	6	17051.560	0.003	17051.255	0.001
			9				8	17051.620	0.001	17051.315	0.003
			8				7	17052.255	0.003	17051.951	0.002
5	2	4	4	4	1	3	3	17504.105	0.001	17500.794	0.008
			6				5	17504.267	0.003	17500.954	0.005
			5				4	17505.005	0.004	17501.687	0.005
5	2	3	5	4	1	4	4	18521.101	0.010		
			6				5	18522.456	0.009	18524.094	0.003
			4				3	18522.824	0.004	18524.459	0.007
9	1	9	8	8	0	8	7	18743.205	0.005	18742.929	0.001
			10				9	18743.253	0.000	18742.976	0.004
			9				8	18743.813	0.004	18743.536	0.000

CHAPTER 6

CONFORMATIONAL LANDSCAPE AND LARGE AMPLITUDE MOTION OF METHYL HEPTANOATE AND METHYL OCTANOATE BY MICROWAVE SPECTROSCOPY

6.1 Introduction

Methyl heptanoate (C₇H₁₃COOCH₃) and methyl octanoate (C₈H₁₅COOCH₃) are volatile compounds that belong to the class of fruit esters, and these substances are important in the food industry as components for perfumes or aromas⁶⁸. Investigation the conformational landscape of these fruit esters in the gas phase may be helpful for understanding the chemical basis of odors because knowing only the chemical formula of a substance will not help to predict its odor. Combinations of volatile compounds with different ratios produce varieties of flavors and scents of fruits⁶⁹, and the gas phase structures of methyl heptanoate and methyl octanoate might provide important information towards understanding the structure-odor relationship.

Since the sense of smell starts from the gas phase molecules, rotational spectroscopy along with the quantum chemical calculations is an ideal technique to investigate these ester molecules. Investigations on the molecules of the methyl alkanoate family have been reported previously: methyl acetate⁷⁰, methyl propionate⁷¹, methyl butyrate⁷², methyl valerate⁶⁸, and methyl hexanoate⁶⁹. The reported methoxy methyl barrier for methyl acetate and methyl propionate molecules are 424.6 cm⁻¹ and 422.8 cm⁻¹, respectively. Microwave spectra were used to determine the structures of two different conformations of methyl butyrate (methyl butanoate)⁷². Two low-energy conformers, one with a fully extended, heavy-atom planar anti/anti structure (*a*, *a*), and the other one with a gauche propyl chain ($g \pm$, *a*) were assigned and the methyl internal rotation of the

methoxy methyl group was found to be $V_3 \approx 420 \text{ cm}^{-1}$. Two conformers of methyl valerate (methyl pentanoate) were also identified from the experimental microwave spectra⁶⁸: the fully extended planar structure C_S and the structure with C₁ symmetry, where the alkyl chain is bent at the γ position of the chain. The barriers to internal rotation of the methoxy methyl group was determined to be 418.059(27) cm⁻¹ and 417.724(70) cm⁻¹ for C_S and C₁ conformer, respectively. The experimental rotational spectra of methyl hexanoate also identified two stable conformers, C_S and C₁, following the pattern of methyl butanoate and methyl pentanoate. The methyl torsional barriers are 417 cm⁻¹ and 415 cm⁻¹ for the C_S and C₁ conformers of methyl hexanoate, respectively. The observed methyl internal rotation barrier decreases continuously with increasing chain length in methyl acetate, methyl propionate, methyl butyrate, methyl valerate, and methyl hexanoate. This observation has been called the "chain length effect" – that the longer alkyl chain, the lower the methyl torsional barrier, until a plateau is reached⁶⁹. Our investigation of methyl heptanoate and methyl octanoate will provide data for longer chains and may contribute to identifying the plateau.

The present study of methyl heptanoate and methyl octanoate (see Figure 1 for molecule numbering) aims to determine the gas phase structures and obtain information on the methyl internal rotation of the methoxy top of the molecules by high resolution rotational spectroscopy along with the computational calculations. We were interested to know how the methyl internal rotation barrier height of the methoxy methyl rotor behaves as the alkyl chains are getting longer. Therefore, this study is important in two aspects: the investigation of the conformational landscape, and the internal dynamics of the methyl rotor.



Figure 6.1: The numbering scheme for (A) Methyl Heptanoate and (B) Methyl Octanoate.

6.2 Experimental and Computational Methods

Rotational spectra of methyl heptanoate and methyl octanoate were recorded in the 9-20 GHz frequency range using the cavity-based FTMW spectrometer described in Chapter 3. Methyl heptanoate (98% pure) was purchased from Aaron chemicals and methyl octanoate (99% pure) was purchased from Sigma-Aldrich. The liquid samples were placed in a reservoir nozzle and heated to 120 - 150 °C using a Watlow band heater and an Omega CN8201 temperature controller to maintain an optimum vapor pressure of the sample. Argon (Ar), at 1.8 atm backing pressure, was flowed over the sample and the gas mixture (sample and Ar) was pulsed into the vacuum chamber using the Series 9 General Valve.

The conformational analysis of methyl heptanoate and methyl octanoate were performed with Gaussian 16 program³⁷ running at Ohio Supercomputer Center³⁸. The MP2 method was used to optimize the starting structures of C_S and C_1 confomers of both molecules. Two different types of basis sets, cc-pvdz and aug-cc-pvtz, were used to optimize C_1 and C_S conformers, respectively, because the studies of methyl valerate⁶⁸ and methyl hexanoate⁶⁹ describe the challenge of optimizing the C_1 and C_S conformers using the same basis set. Those studies showed that a soft degree of freedom around the bond connecting the carbonyl group and the alkyl chain makes these molecules extremely flexible, and different basis sets predict remarkably different structures and rotational constants (differences up to 500MHz)⁶⁹. The MP2/cc-pVDZ level yielded the best match between the predicted and experimental rotational constants, and was used to optimize the C_1 conformer of methyl heptanoate and methyl octanoate.

The programs SPCAT and SPFIT were used to predict and fit the rotational spectra of methyl heptanoate and methyl octanoate conformers⁷³. The program PGOPHER⁷⁴ was used visualize and compare the simulated and experimental spectra. Global fits of the rotational

constants, centrifugal distortion constants, nuclear quadrupole coupling constants, and A and E tunneling states were performed using XIAM⁵⁵.



Figure 6.2: Portion of the microwave spectrum of methyl heptanoate (C_S conformer) showing (*A*) and (*E*) tunneling state components of the 10 $_{1 10}$ - 9 $_{0 9}$ rotational transition with 2000 shots averaged.



Figure 6.3: Portion of the microwave spectrum of methyl heptanoate (C_1 conformer) showing (A) and (E) tunneling state components of the 17 $_{117}$ - 16 $_{116}$ rotational transition with 400 shots averaged.



Figure 6.4: Portion of the microwave spectrum of methyl octanoate (C_s conformer) showing (*A*) and (*E*) tunneling state components of the 12 $_{1\,12}$ -11 $_{0\,11}$ rotational transition with 2000 shots averaged.



Figure 6.5: Portion of the microwave spectrum of methyl octanoate (C_1 conformer) showing (*A*) and (*E*) tunneling state components of three different rotational transitions with 5000 shots averaged.

6.3 Results

The full geometry optimization of C_S and C_1 conformers were performed using MP2/augcc-pVDZ and MP2/aug-cc-pVTZ levels of theory, respectively for both methyl heptanoate and methyl octanoate molecules. As found for the previous studies of methyl butyrate⁷², methyl valerate⁶⁸, and methyl hexanoate⁶⁹, the two lowest-energy conformers are C_S (one with a fully extended heavy-atom planar anti/anti structure (a/a)) and C_1 (with a gauche chain where the β carbon is slightly tilted out of the plane). The optimized structures for methyl heptanoate and methyl octanoate are shown in figures 6.6 and 6.7, respectively.

Rotational spectra were measured and assigned to the C_S and C_1 conformers of methyl heptanoate and methyl octanoate. 10 rotational transitions for C_S conformer and 28 rotational transitions for C_1 conformer were assigned for methyl heptanoate. For methyl octanoate, 11 rotational transitions and 46 rotational transitions were assigned for C_S and C_1 conformers, respectively. Final fitting of assigned transitions for each conformer of both molecules was carried out using XIAM⁵⁵ and the *A*-reduction Hamiltonian. The barriers to methyl internal rotation for C_S and C_1 conformers were found to be 399.4(10) cm⁻¹ and 411(4) cm⁻¹ for methyl heptanoate and 399.2(13) cm⁻¹ and 409.3(26) cm⁻¹ for methyl octanoate, respectively. The comparison between the experimentally determined and theoretically calculated rotational spectroscopic parameters for methyl heptanoate and methyl octanoate are given in tables 6.1 and 6.2. Table 6.3 compares the second moments of C_S and C_1 conformers of methyl heptanoate and methyl octanoate. The experimentally determined values for second moments unambiguously confirm the assignment of the spectra.



Figure 6.6: The optimized structures of the two assigned conformers of methyl heptanoate, C_S and C_1 conformers.



Figure 6.7: The optimized structures of the two assigned conformers of methyl octanoate, C_S and C_1 conformers.

Parameter	C1 Conformer		C _s Conformer	
	Experimental	Theoretical	Experimental	Theoretical
	(XIAM)	(MP2/aug-cc-pVDZ)	(XIAM)	(MP2/aug-cc-pVTZ)
A/MHz	3260.993(7)	3263.5	6333.7(5)	6366.3
<i>B</i> /MHz	424.101(11)	424.2	336.1(2)	338.5
C/MHz	401.891(12)	400.9	323.84(4)	325.9
⊿ _J /kHz	0.077(13)		-0.3(5)	
<i>∆_{JK}</i> /kHz	-3.02(4)		Fixed	
Δ_k/kHz	36.8(5)		Fixed	
$V_3 / { m cm}^{-1}$	411(4)		399.4(10)	
⊿v _{rms} /kHz	12.6		3.7	
Ν	28		10	

Table 6.1: Comparison between experimentally determined and theoretically calculated rotational spectroscopic parameters for methyl heptanoate.

Parameter	C ₁ Conformer		C _S Conformer	
	Experimental	Theoretical	Experimental	Theoretical
	(XIAM)	(MP2/aug-cc-pVDZ)	(XIAM)	(MP2/aug-cc-pVTZ)
A/MHz	2657.28(3)	2650.6	5969.1(5)	6008.3
<i>B</i> /MHz	306.8750(5)	306.9	241.99(12)	243.9
C/MHz	290.4743(4)	289.9	235.53(5)	237.1
⊿ _J /kHz	0.0382(5)		0.4(3)	
<i>∆_{JK}</i> /kHz	-1.708(3)		Fixed	
Δ_k/kHz	35(6)		Fixed	
$V_3 / \text{ cm}^{-1}$	409.3(26)		399.2(13)	
⊿v _{rms} /kHz	13.4		3.7	
Ν	46		11	

Table 6.2: Comparison between experimentally determined and theoretically calculated rotational spectroscopic parameters for methyl octanoate.

Table 6.3. Comparison of theoretical (T) and experimental (E) values of the second moments of inertia for C_s and C1 conformers of methyl heptanoate and methyl octanoate.

Parameter	Methyl Heptanoate			Methyl Octanoate					
	C _s conformer		C ₁ conformer		C _s conformer		C ₁ conformer		
	Т	E	Т	Е	Т	E	Т	E	
P_{aa} / amu Å ²	1482.182	1492.539	1148.481	1147.090	2059.925	2074.710	1599.713	1598.281	
P_{bb} / amu Å ²	68.524	68.236	111.989	110.416	71.705	70.956	143.471	141.585	
P_{cc} / amu Å ²	10.858	11.565	42.867	43.235	12.407	13.710	47.196	48.602	

6.4 Discussion

The rotational spectra of both molecules, methyl heptanoate and methyl octanoate, were recorded and assigned to the C_S and C₁ conformers. Independent of the length of the alkyl chain, the C_S conformer is always observed showing an all-anti geometry. The C₁ conformer has a configuration where the β -carbon is slightly tilted out of the plane (shown in Figure 1). The previous studies, methyl butyrate⁷², methyl valerate⁶⁸, and methyl hexanoate⁶⁹, also exhibit this structural behavior, whereas methyl acetate and methyl propionate are not sufficiently long to show such a structural behavior.

The experimentally determined rotational constants are in excellent agreement with the theoretically calculated rotational constants in C_S and C_1 conformers of both molecules (Table 6.1 and 6.2); the experimental rotational constants, A, B, and C of methyl heptanoate are within 0.5%, 0.7%, and 0.6% for the C_S conformer, and 0.08%, 0.02%, and 0.2% for the C_1 conformer, respectively. For methyl octanoate, A, B, and C within 0.7%, 0.8%, and 0.7% for C_S conformer and 0.3%, 0.01%, and 0.2% for C_1 conformer, respectively.

Second moments of inertia, P_{aa} , P_{bb} , and P_{cc} , provide a more reliable means of the conformational assignments. The second moments along the principal axes are given by

$$P_{aa} = \sum m_i a_i^2 = \frac{1}{2} (-I_a + I_b + I_c),$$

$$P_{bb} = \sum m_i b_i^2 = \frac{1}{2} (I_a - I_b + I_c), \text{ and}$$

$$P_{cc} = \sum m_i c_i^2 = \frac{1}{2} (I_a + I_b - I_c)$$

where the m_i are the atomic masses, a_i , b_i , and c_i are the atomic coordinates along the a, b, and c principal axes, and I_a , I_b , and I_c are the principal moments of inertia, respectively. Table 6.3 provides the comparison of theoretical and experimental values of the second moments of inertia

for C_S and C1 conformers of methyl heptanoate and methyl octanaoate, and confirm the conformational assignments.

The theoretical models for C_S and C_1 conformers of both methyl heptanoate and methyl octanoate were reproduced by the experimentally determined rotational constants. Therefore, the theoretical structures can be considered as the experimental structures, and no further structural fittings are necessary. The coordinates for optimized structures of each conformer of methyl heptanoate and methyl octanoate are given in tables 6.5, 6.6, 6.7, and 6.8 and the frequencies of all resolved transitions are available in tables 6.9, 6.10, 6.11, and 6.12.

The structural optimization of methyl heptanoate and methyl octanoate was performed by using MP2 method and two different basis sets for C_S and C₁ conformers, MP2/aug-cc-pVTZ for C_S and MP2/aug-cc-pVDZ for C₁. The MP2/aug-cc-pVTZ level of theory was used for the better prediction of the theoretical rotational constants of the C_S conformer of both molecules. The MP2/aug-cc-pVTZ level of theory failed to predict the reliable rotational constants close to the assigned C₁ rotational constants. As the previous studies, methyl butyrate⁷², methyl valerate⁶⁸, and methyl hexanoate suggest MP2/aug-cc-pVDZ level of theory was employed for the theoretical calculation to address the so-called "theta problem" for C₁ conformer of both molecules. The soft degree of freedom around the C_{α} and C_{β} bond makes the molecules extremely flexible and therefore, the calculated model rotational constants with MP2/aug-cc-pVTZ were significantly far from the experimental rotational constants.

The barrier to methyl internal rotation of methoxy methyl group is 399.4(10) cm⁻¹ for C_s and 411(4) cm⁻¹ for C₁ conformer of methyl heptanoate and for the methyl octanoate, 399.2(13) cm⁻¹ and 409.3(26) cm⁻¹ for C_s and C₁ conformers, respectively. These experimental values of both C_s and C₁ conformers follow the same slight decreasing trend in the barrier height when

increasing the length of the alkyl chain of the molecule. Table 6.4 shows the experimental barriers to methyl internal rotation for other previously investigated molecules of the methyl alkynoate series. However, there are no sufficient evidence to claim that the molecular conformation and the length of the alkyl chain significantly affect the barrier height of the methoxy methyl group.

Table 6.4: Methyl alkynoates and their respective torsional barriers (V_3) of methoxy methyl group.

Molecule	Cs conformer (cm ⁻¹)	C ₁ conformer (cm ⁻¹)
Methyl acetate ⁷⁰	424.581(56)	
Methyl propionate ⁷¹	422.801(22)	
Methyl butyrate ⁷²	420.155(71)	418.447(59)
Methyl valerate ⁶⁸	418.059(27)	417.724(70)
Methyl hexanoate ⁶⁹	416.890(96)	415.15(13)
Methyl heptanoate [this work]	399.4(10)	411(4)
Methyl Octanoate [this work]	399.2(13)	409.3(26)

6.5 Conclusion

The rotational spectrum of methyl heptanoate and methyl octanoate were recorded in the 9-18 GHz frequency range. The spectra of both molecules were assigned for two lowest-energy C_1 and C_S conformers. The MP2/aug-cc-pVTZ level of theory failed to calculate reliable rotational constants to guide the assignment of C_1 conformer, while the MP2/aug-cc-pVDZ level succeeded. XIAM was employed to do the final fitting for C_1 and C_2 conformers of both species. The experimental structures were assigned to the theoretically calculated model structures, as the theoretical and experimental rotational constants were in great agreement. The barrier to methyl internal rotation of methoxy methyl group is 399.4(10) cm⁻¹ for C_5 and 411(4) cm⁻¹ for C_1 conformer of methyl heptanoate and for the methyl octanoate, 399.2(13) cm⁻¹ and 409.3(26) cm⁻¹ for C_5 and C_1 conformers, respectively.

6.6 Optimized Molecular Coordinates for Methyl Heptanoate conformers and Methyl Octanoate conformers

Table 6.5: Optimized molecular structure coordinates (Å) for C_S conformer of methyl heptanoate.

Atom Number	Atom	a	b	с
1	С	-5.316456	0.399008	-0.002337
2	С	-4.082350	-0.494082	0.001146
3	Н	-5.328102	1.045991	0.875936
4	Н	-5.327720	1.039652	-0.885250
5	С	-2.786375	0.306334	-0.001452
6	Н	-4.099512	-1.151189	-0.872343
7	Н	-4.099895	-1.144883	0.879335
8	С	-1.541814	-0.571600	0.001974
9	Н	-2.766321	0.964916	0.872584
10	Н	-2.765953	0.958610	-0.880197
11	С	-0.250987	0.236535	-0.000613
12	Н	-1.562390	-1.230168	-0.872328
13	Н	-1.562756	-1.223849	0.880994
14	С	0.978379	-0.655658	0.002789
15	Н	-0.222491	0.894465	0.870262
16	Н	-0.222216	0.888265	-0.876163
17	С	2.267300	0.120815	0.001072
18	Н	0.988101	-1.316954	-0.866742
19	Н	0.987289	-1.310664	0.877158
20	0	3.331240	-0.712690	-0.002070
21	С	4.601451	-0.041854	-0.002815
22	Н	4.698599	0.582581	-0.887728
23	Н	5.344947	-0.831063	-0.005337
24	Н	4.701296	0.579457	0.883999
25	0	2.369382	1.328933	0.002641
26	Н	-6.236893	-0.184644	-0.000437

Atom Number	Atom	a	b	с
1	С	4.787515	-0.679567	0.086251
2	С	3.358440	-0.705197	-0.461187
3	Н	5.254286	0.306971	-0.078484
4	Н	4.795636	-0.875866	1.172284
5	С	2.452915	0.339894	0.195688
6	Н	2.919234	-1.709141	-0.310122
7	Н	3.375374	-0.534915	-1.554287
8	С	1.019192	0.318218	-0.339941
9	Н	2.888258	1.347288	0.044070
10	Н	2.434344	0.170618	1.289893
11	С	0.119365	1.364203	0.324418
12	Н	0.587714	-0.686345	-0.179809
13	Н	1.035424	0.492179	-1.434052
14	С	-1.310671	1.354675	-0.216715
15	Н	0.552826	2.369592	0.173538
16	Н	0.089028	1.181705	1.413093
17	С	-2.071699	0.105275	0.179800
18	Н	-1.332888	1.442231	-1.316600
19	Н	-1.888584	2.213466	0.171156
20	0	-3.206630	-0.016370	-0.553495
21	С	-4.003453	-1.158609	-0.199345
22	Н	-3.436492	-2.090705	-0.345810
23	Н	-4.874134	-1.129053	-0.866531
24	Н	-4.321766	-1.102629	0.853006
25	0	-1.744692	-0.682345	1.047737
26	Н	5.422688	-1.439031	-0.399092

Table 6.6: Optimized molecular structure coordinates (Å) for C_1 conformer of methyl heptanoate.

Atom Number	Atom	a	b	с
1	С	-6.025645	-0.258050	0.000242
2	С	-4.721843	0.530416	-0.002881
3	Н	-6.089761	-0.902015	-0.877758
4	Н	-6.894109	0.400055	-0.002695
5	Н	-6.090058	-0.894545	0.883651
6	С	-3.495338	-0.373310	0.001176
7	Н	-4.685000	1.187613	0.869945
8	Н	-4.684695	1.180170	-0.881248
9	С	-2.182862	0.399435	-0.001880
10	Н	-3.529928	-1.032076	-0.872647
11	Н	-3.530234	-1.024616	0.880560
12	С	-0.957590	-0.505542	0.002203
13	Н	-2.147824	1.057765	0.871771
14	Н	-2.147511	1.050285	-0.881108
15	С	0.350455	0.274470	-0.000831
16	Н	-0.992539	-1.164175	-0.871625
17	Н	-0.992850	-1.156689	0.881614
18	С	1.560191	-0.644154	0.003279
19	Н	0.393042	0.932315	0.869571
20	Н	0.393287	0.924924	-0.876799
21	С	2.865842	0.104485	0.001232
22	Н	1.555681	-1.306209	-0.865737
23	Н	1.554852	-1.298610	0.878127
24	0	3.911361	-0.751831	-0.002399
25	С	5.195835	-0.109184	-0.003194
26	Н	5.306732	0.513408	-0.887853
27	Н	5.922023	-0.914368	-0.006242
28	Н	5.309816	0.509453	0.883852
29	0	2.993807	1.310113	0.003179

Table 6.7: Optimized molecular structure coordinates (Å) for C_S conformer of methyl octanoate.

Atom Number	Atom	a	b	с
1	С	5.397893	1.106229	0.268849
2	С	4.389660	0.093756	-0.279623
3	Н	5.088461	2.138075	0.028713
4	Н	6.404008	0.948049	-0.153692
5	Н	5.475993	1.027747	1.367018
6	С	2.982036	0.290059	0.289967
7	Н	4.732508	-0.933464	-0.052923
8	Н	4.347629	0.170093	-1.382479
9	С	1.962277	-0.715534	-0.250770
10	Н	2.635905	1.317249	0.062801
11	Н	3.021466	0.213557	1.394348
12	С	0.555349	-0.509996	0.316429
13	Н	2.305448	-1.743515	-0.021835
14	Н	1.923090	-0.638652	-1.354594
15	С	-0.460543	-1.516633	-0.231105
16	Н	0.215417	0.514372	0.079620
17	Н	0.591451	-0.591187	1.420826
18	С	-1.864508	-1.322836	0.342711
19	Н	-0.118937	-2.542791	-0.003586
20	Н	-0.509627	-1.424957	-1.330475
21	С	-2.516685	-0.043350	-0.142034
22	Н	-1.858243	-1.314675	1.446257
23	Н	-2.533514	-2.151180	0.045725
24	0	-3.610161	0.249242	0.605785
25	С	-4.306596	1.429531	0.173373
26	Н	-3.649243	2.311048	0.223454
27	Н	-5.153302	1.541341	0.862345
28	Н	-4.663881	1.315987	-0.861693
29	0	-2.145673	0.632724	-1.083263

Table 6.8: Optimized molecular structure coordinates (Å) for C_1 conformer of methyl octanoate.

6.7 Assigned transition frequencies of methyl heptanoate and methyl octanoate

J′	K'_a	K_c'	J″	$K_a^{\prime\prime}$	$K_c^{\prime\prime}$	Tunneling	Obs	Obs-calc
						State	(MHz)	(MHz)
6	1	6	5	0	5	А		
						E	9834.2080	0.002
7	1	7	6	0	6	А	10453.2120	0.004
						E	10451.8830	0.001
9	1	9	8	0	8	А	11670.7960	0.008
						E	11669.6648	0.003
10	1	10	9	0	9	А	12271.0850	0.003
						E	12270.0030	0.001
11	1	11	10	0	10	А	12865.8580	0.004
						E	12864.8160	0.004
13	1	13	12	0	12	А	14039.3945	0.000
						Е	14038.4100	0.004
14	1	14	13	0	13	А	14618.4822	0.001
						Е	14617.5210	0.003
15	1	15	14	0	14	А		
						E	15191.7430	0.002
16	1	16	15	0	15	А	15762.1765	0.000
						Е	15761.2570	0.000
17	1	17	16	0	16	А	16327.1811	0.000
						E	16326.2770	0.001

Table 6.9: Assigned transition frequencies for C_s conformer of methyl heptanoate.

J′	K'_a	K_c'	J''	$K_a^{\prime\prime}$	$K_c^{\prime\prime}$	Tunneling	Obs	Obs-calc
						State	(MHz)	(MHz)
12	0	12	11	0	11	А	9874.9673	0.004
						Е	9874.9457	0.005
10	1	10	9	0	9	А	10525.4327	0.006
						Е	10525.5026	0.009
13	1	13	12	1	12	А	10582.2488	0.007
						E	10582.2354	0.001
13	0	13	12	0	12	А	10691.1773	0.005
						E	10691.1564	0.006
9	1	8	8	0	8	А	10798.4092	0.029
						E	10798.2663	0.014
14	1	14	13	1	13	А	11394.3918	0.007
						E	11394.3802	0.002
14	0	14	13	0	13	А	11505.9402	0.004
						Е	11505.9180	0.005
14	2	13	13	2	12	А	11554.0915	0.014
						E	11554.0266	0.006
14	1	13	13	1	12	А	11702.9377	0.012
						Е	11702.8948	0.011
15	1	15	14	1	14	А	12206.1623	0.007
						E	12206.1510	0.003
15	0	15	14	0	14	А	12319.2276	0.004
						Е	12319.2059	0.006
15	2	13	14	2	12	А	12447.3043	0.019
						E	12447.2038	0.014
15	1	14	14	1	13	А	12536.0122	0.013

Table 6.10: Assigned transition frequencies for C_1 conformer of methyl heptanoate.

						E	12535.9673	0.013
13	1	13	12	0	12	А	12663.6176	0.022
						E	12663.6545	0.030
11	1	10	10	0	10	А	12707.4206	0.014
						E	12707.2486	0.013
16	1	16	15	1	15	А	13017.5365	0.020
						Е	13017.5490	0.015
16	0	16	15	0	15	А	13131.0368	0.003
						Е	13131.0146	0.006
12	1	11	11	0	11	А	13685.1377	0.006
						Е	13684.9485	0.011
17	1	17	16	1	16	А	13828.5314	0.018
						Е	13828.5437	0.017
6	3	3	6	2	5	А	14240.7035	0.002
						Е	14240.7589	0.004
7	3	4	7	2	6	А	14243.0304	0.002
						Е	14243.0616	0.001
8	3	5	8	2	7	А	14246.4626	0.002
						Е	14246.4815	0.003
9	3	6	9	2	8	А	14251.3340	0.000
						Е	14241.3425	0.004
10	3	7	10	2	9	А	14258.0137	0.007
						Е	14258.0137	0.000
18	1	18	17	1	17	А	14639.1271	0.016
						Е	14639.1378	0.018
13	1	12	12	0	12	А	14679.4447	0.001
						Е	14679.2354	0.007
18	0	18	17	0	17	А	14750.3448	0.002
						Е	14750.3231	0.005

J′	K'a	K'c	J''	$K_a^{\prime\prime}$	<i>K</i> _c ''	Tunneling	Obs	Obs-calc
						State	(MHz)	(MHz)
8	1	8	7	0	7	А	9434.5240	0.002
						E	9433.1420	0.001
9	1	9	8	0	8	А	9883.0300	0.004
						E	9881.7670	0.001
10	1	10	9	0	9	А	10328.3800	0.002
						E	10327.2100	0.004
11	1	11	10	0	10	А	10770.6300	0.002
						E	10769.5300	0.001
12	1	12	11	0	11	А	11209.8010	0.005
						E	11208.7620	0.001
13	1	13	12	0	12	А	11645.9670	0.007
						E	11644.9550	0.002
14	1	14	13	0	13	А	12079.1370	0.002
						E	12078.1747	0.004
15	1	15	14	0	14	А	12509.3930	0.006
						E	12508.4592	0.000
16	1	16	15	0	15	А	12936.8000	0.002
						E	12935.8820	0.000
17	1	17	16	0	16	А	13361.4000	0.000
						E	13360.5060	0.001
18	1	18	17	0	17	А	13783.2760	0.002
						E	13782.3940	0.002

 Table 6.11: Assigned transition frequencies for C_S conformer of methyl octanoate.

J'	K'a	K_c'	J″	$K_a^{\prime\prime}$	$K_c^{\prime\prime}$	Tunneling	Obs	Obs-calc
						State	(MHz)	(MHz)
16	1	16	15	1	15	А	9412.7783	0.002
						Е	9412.7715	0.002
11	1	10	10	0	10	А	9497.6373	0.005
						Е	9497.5451	0.002
16	0	16	15	0	15	А	9501.2964	0.000
						Е	9501.2819	0.002
16	2	15	15	2	14	А	9547.8492	0.025
						Е	9547.8910	0.006
16	6	10	15	6	9	А	9560.8518	0.001
						Е	9560.8331	0.001
16	5	12	15	5	11	А	9561.0187	0.002
						Е	9560.9982	0.004
16	4	13	15	4	12	А	9561.9699	0.001
						Е	9561.9782	0.006
16	3	14	15	3	13	А	9563.8485	0.002
						Е	9564.6079	0.013
16	3	13	15	3	12	А	9566.2163	0.003
						Е	9565.4199	0.013
16	2	14	15	2	13	А	9603.5322	0.016
						Е	9603.4714	0.014
16	1	15	15	1	14	А	9672.5971	0.007
						E	9672.5579	0.011
17	1	17	16	1	16	А	9999.3473	0.003
						Е	9999.3403	0.003
17	0	17	16	0	16	А	10088.1110	0.001
						Е	10088.0960	0.002
17	2	16	16	2	15	А	10143.1990	0.016

Table 6.12: Assigned transition frequencies for C_1 conformer of methyl octanoate.

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						Е	10143.2080	0.018
17	3	14	16	3	13	А	10162.3780	0.005
						Е	10164.5000	0.019
17	1	16	16	1	15	А	10274.6800	0.002
						Е	10274.6510	0.006
18	1	18	17	1	17	А	10585.6370	0.005
						E	10585.6390	0.005
18	0	18	17	0	17	А	10673.9170	0.002
						Ε	10673.9030	0.000
18	2	17	17	2	16	А	10738.2840	0.020
						E	10738.2860	0.021
18	6	12	17	6	11	А	10756.2870	0.012
						Е	10756.2390	0.015
18	6	13	17	6	12	А	10756.2870	0.012
						E	10756.2390	0.019
18	7	11	17	7	10	А	10756.4395	0.012
						Е	10756.3930	0.013
18	7	12	17	7	11	А	10756.4395	0.012
						Е	10756.3930	0.018
18	5	14	17	5	13	А	10756.7030	0.013
						E	10756.6560	0.017
18	5	13	17	5	12	А	10756.7030	0.013
						E	10756.6560	0.013
18	2	16	17	2	15	А	10815.7330	0.021
						E	10815.6850	0.019
18	1	17	17	1	16	А	10876.2850	0.005
						E	10876.2430	0.011
13	1	12	12	0	12	А	10925.5850	0.001
						E	10925.4760	0.006
19	0	19	18	0	18	А	11258.7480	0.003
						E	11258.7350	0.002

19	5	14	18	5	13	А	11354.5990	0.001
						Е	11354.5730	0.002
19	2	17	18	2	16	А	11422.8710	0.014
						Е	11422.8300	0.016
19	1	18	18	1	17	А	11477.3350	0.006
						E	11477.3040	0.009
8	2	7	7	1	6	А	11625.0710	0.013
						Е	11623.0980	0.024
14	1	13	13	0	13	А	11657.9610	0.003
						E	11657.8450	0.006
20	1	20	19	1	19	А	11757.4230	0.011
						Е	11757.4120	0.009
20	0	20	19	0	19	А	11842.6560	0.004
						Е	11842.6420	0.002
20	2	19	19	2	18	А	11927.6330	0.037
						Е	11927.6440	0.019
20	2	18	19	2	17	А	12030.6430	0.029
						Е	12030.5900	0.017
20	1	19	19	1	18	А	12077.8100	0.009
						Е	12077.7770	0.013
9	2	7	8	1	7	А	12185.2440	0.001
						E	12186.2020	0.040
15	1	14	14	0	14	А	12403.4190	0.003
						Е	12403.2910	0.006
16	1	15	15	0	15	А	13162.5570	0.001
						E	13162.4090	0.002
11	2	9	10	1	9	А	13257.9390	0.005
						Е	13258.3220	0.003
12	2	10	11	1	10	А	13790.2210	0.010
						E	13790.4560	0.016
17	1	16	16	0	16	А	13935.9390	0.004

						E	13935.7770	0.002
13	2	11	12	1	11	А	14321.1360	0.017
						E	14321.2740	0.030

CHAPTER 7

INTRAMOLECULAR HYDROGEN-BONDING MOTIF AND MOLECULAR STRUCTURE IN THE 2-METHYLAMINOETHANOL-WATER COMPLEX

7.1 Introduction

Hydrogen bonding plays a predominant role in stabilizing the large-scale structures in biological systems^{31,75}, and investigations of hydrogen-bonding motifs elucidates the details of these interactions, and the conformational behavior of molecules and molecular complexes. The structural sensitivity of molecular moments of inertia makes rotationally resolved spectroscopy an ideal technique for these investigations; rotational spectra have been used to obtain precise structures and investigate tunneling dynamics of intermolecular hydrogen-bonded complexes^{31,76,77}.

We have used rotational spectroscopy to identify changes in molecular structures upon formation of new hydrogen bonding networks. The prototypical molecule 2-aminoethanol has an intramolecular hydrogen bond from alcohol to amine characterized by a 2.808 Å O····N separation and 55.4° O – C – C – N dihedral angle⁷⁸. Formation of the 2-aminoethanol-water complex is accompanied by forming an additional network of hydrogen bonds from the hydroxyl to the water oxygen and from water to the amino nitrogen⁷⁹. The structure of the 2-aminoethanol monomer adjusts, with the O····N separation and O – C – C – N dihedral angle increasing to 3.100 Å and 75° respectively. The hydroxyl-to-oxirane-oxygen hydrogen bond in glycidol (2-oxiranemethanol) also changes to accommodate the formation of the new network of hydrogen bonds in the glycidolwater complex³¹.
We now extend these investigation to the rotational spectrum and the structure of the hydrogen-bonded complex 2-(methylamino) ethanol-water (2-MAE-water). In this complex a methyl group disrupts formation of a possible hydrogen-bonding network by replacing one of the N-H sites of 2-aminoethanol. The rotational spectrum of 2-MAE monomer was used to identify two different conformations in the gas phase, increasing the number of possible structures for the water complex^{80,81}.

The 2-MAE monomer conformers orient the hydroxyl and amine groups gauche (G) or gauche' (G') about the ethanolic C-C bond with the methyl amine either gauche (G) or trans (T) from the ethanolic C-C bond^{80,81}; see Figure 7.1. Both conformers have an intramolecular hydrogen bond from the hydroxyl to the amino nitrogen. The GG conformation was calculated to be 0.65 (MP2/6-311++G(d,p)) to 1.32 (B3LYP/6-311++G(d,p))) kJ mol⁻¹ higher in energy than the G'T conformer⁸¹. A cavity-based FTMW spectrometer was used to resolve spectral splittings arising from methyl internal-rotation tunneling, and the fitted values of the V₃ barriers were found to be 11.91 and 9.7 kJ mol⁻¹ for the G'T and GG conformers respectively⁸¹.



G'T



Figure 7.1. Structures of the conformers of 2-MAE described in reference ⁸¹

7.2 Experimental and Computational Methods

The rotational spectrum of 2-MAE-water was recorded with the cavity-based FTMW spectrometer described in Chapter 3. The liquid sample of 2-MAE (\geq 98%, TCI) purchased from Sigma Aldrich, was placed in the sample reservoir nozzle without further purification and heated to 45 °C using a Watlow band heater and an Omega CN8201 temperature controller to vaporize the sample. Argon gas was used as the carrier gas at 1.5 atm backing pressure. A glass bulb with a stem filled with1 mL of water was attached to the gas manifold to introduce water vapor to the system. The gas mixture (sample vapor+water vapor+Ar) was pulsed into the vacuum chamber using the Series 9 General Valve. The expansion is parallel to the cavity axis which causes Doppler splittings of approximately 60 kHZ for each transition with typical FWHM of each Doppler component of 13 kHZ.

Avogadro molecular modelling software was used to create different orientations of the 2-MAE-water complex, and the starting structures were optimized using the MP2/6-311++G(d,p) level of theory. All calculations were performed using GAUSSIAN 16 program³⁷ running on the OWENS cluster at the Ohio Supercomputer Center (OSC). The predicted orientations of the 2-MAE-water complex were named according to the dihedral orientations of the backbone of the monomer, O - C - C - N dihedral angle (τ) and C - C - N - C dihedral angle (χ). The programs RRFIT (rigid-rotor fitting), ZFAP (rotational and centrifugal distortion fitting), and QUAD2I (nuclear quadrupole hyperfine fitting), were used to assign the experimental spectrum of 2-MAEwater complex. The estimated methyl barrier for N – CH₃ rotor was calculated using relaxed potential energy scan at B3LYP/6-311++G(d,p) level, in 10° steps while optimizing all remaining parameters.



Figure 7.2. Structures of the lowest-energy model conformers of 2-MAE-water complexes at the MP2/6-311++G(d,p) level. O – C – C – N dihedral angle (τ) and C – C – N – C dihedral angle (χ).

7.3 Results

Models of the 2-MAE monomer conformations were created using MP2/6-311++G(d,p) calculations for comparison to previous spectroscopic investigations^{80,81} and further structural analysis; these models are summarized in Table 7.1. For 2-MAE, the G'T conformation was calculated to be the lowest energy structure, with the GG conformation 0.65 kJ mol⁻¹ higher in energy. The relative energies of the conformers were within 1 kJ mol⁻¹ of the previously calculated energies⁸¹. The rotational constants calculated from these model structures were within 1.5% of the experimental values for 2-MAE G'T and within 0.5% of the experimental values for 2-MAE GG⁸¹. Both the G'T and GG conformers of 2-MAE have a gauche configuration of O – C – C – N, with $\tau = 57.7^{\circ}$ and 54.5° respectively, and O…N distances of 2.809 Å and 2.787 Å respectively. This structure facilitates formation of an intramolecular hydrogen bond from the hydroxyl group to the amino nitrogen in both conformers.

Table 7.1. Ab initio model parameters for MAE monomer conformations.

Parameter	2-MAE						
	G'T	GG					
$\Delta E / \text{kJ mol}^{-1}$	0.00	0.65					
O…N / Å	2.809	2.787					
$\tau/^{\circ}$ (OCCN)	57.7	54.5					

Ab initio models of the structures of the 2-MAE-H₂O complexes were made at the MP2/6-311++G(d,p) level. Starting structures for optimization of the complexes were created from each of the observed monomer conformations with water placed in different locations around the hydroxyl or amine groups.

Four stable structures were found for 2-MAE-H₂O; these models are summarized in Table 7.2 and shown in Figure 7.2. The lowest energy structure (Complex I) is derived from the G'T conformation of 2-MAE with new network of hydrogen bonds from the water to the amino nitrogen and from the hydroxyl to the water oxygen – similar to the hydrogen-bonding network found in the 2-aminoethanol-water complex⁷⁹. The higher energy model structures disrupt the intramolecular hydrogen bond of the 2-MAE monomer (Complex III and Complex IV) or have water only acting as a hydrogen bond donor to the hydroxyl oxygen (Complex II).

Parameter	Complex I	Complex II	Complex III	Complex IV
$\Delta E / \text{kJ mol}^{-1}$	0	5.24	13.04	24.71
A/MHz	3397.09	4093.70	3190.16	6308.80
<i>B</i> /MHz	2320.41	1663.49	2401.43	1189.95
C/MHz	1490.61	1393.04	1474.05	1066.48
$\mu_a/{ m D}$	-1.42	-0.69	-0.02	0.98
$\mu_b/{ m D}$	0.64	0.36	-1.20	-0.41
$\mu_c/{ m D}$	-0.60	0.60	1.70	0.18
ΔI_{rms} / amu Å ²	6.4	53.7	11.4	149.8
$\tau/^{\circ}$ (OCCN)	-76.5	-55.5	-69.1	-70.7
O…N∕Å	3.119	2.765	2.952	2.995
II hand / Å	a = 1.845	a = 1.881	a = 1.949	a = 1.939
H bond / A	b = 1.896			
Monomer	G'T	G'T	G'T	GG

Table 7.2. Theoretically Calculated Spectroscopic Parameters for 2-MAE-H₂O complex at MP2/6- 311++G(d,p) level of theory.

The rotational spectrum of 2-MAE-water was recorded and assigned to a single conformational structure of the complex. The experimentally determined rotational spectroscopic parameters are given in the Table 7.3. 23 rotational transitions were fit to Watson's A-reduced Hamiltonian: A=3368.071(5) MHz, B=2282.618(2) MHz, and C=1537.989(13) MHz and the resolved ¹⁴N nuclear hyperfine components were fit the nuclear quadrupole coupling constants: $\chi_{aa} = 1.547(5)$ MHz and $\chi_{bb} = -1.068(5)$ MHz. Well resolved potions of the rotational spectrum of 2-MAE-H₂O are shown in Figures 7.3 and 7.4. The assigned rotational transitions and their observed frequencies are listed in Table 7.5. No methyl internal rotation tunneling splittings were observed in the spectrum, suggesting a higher methyl internal rotation barrier for the methylamino methyl group. The estimated methylamino methyl rotor (N – CH₃) barrier is 1029.3 cm⁻¹ and the relaxed potential energy scan diagram is shown in the Figure 7.5.

Table 7.3. Experimentally determined rotational spectroscopic parameters for 2	2-
MAE-water complex.	

<i>B</i> /MHz	2282.618(2)
C/MHz	1537.989(13)
⊿ _J /kHz	0.85(4)
Δ_{JK}/kHz	1.1(2)
Δ_k/kHz	4.2(10)
χ_{aa} / MHz	1.547(5)
χ_{bb}/MHz	-1.068(5)
$\Delta v_{rms}/kHz$	4.9
Ν	23



Figure 7.3. Portion of the microwave spectrum of 2-MAE-H₂O complex showing nuclear quadrupole hyperfine components of the 3_{12} - 2_{11} rotational transition with 5000 shots averaged.



Figure 7.4. Portion of the microwave spectrum of 2-MAE-H₂O complex showing nuclear quadrupole hyperfine components of the 4_{13} - 3_{12} rotational transition with 5000 shots averaged.



Figure 7.5. Potential energy scan at B3LYP/6-311++G(d,p) for $N - CH_3$ methyl rotor.

7.4 Discussion

The first experimental scans of 2-MAE monomer have been recorded and assigned two rotameric forms (G'T and GG) of the molecule in 1975⁸⁰. Later, the molecule was revisited and recorded the experimental rotational spectra for the two most stable conformers of 2-MAE, resulting the similar rotational constants for G'T conformer and slightly different A rotational constant for the GG conformer⁸¹. The assigned rotational constants, A, B, and C for G'T conformer are 12123.7430(6) MHz, 2653.8058(2) MHz, and 2400.7362(2) MHz and for GG conformer, 9155.314(2) MHz, 3076.3199(5) MHz, and 2868.6381(4), respectively.

The MP2/6-311++G)d,p) optimized four structures of the 2-MAE-H₂O complex are within 25 kJ mol⁻¹ compared to the lowest energy structure of the complex. The second lowest energy structure, complex II is 5.24 kJ mol⁻¹ higher in energy than the lowest energy complex, and the third and fourth complexes are 13.04 kJ mol⁻¹ and 24.71 kJ mol⁻¹ higher in energy than the lowest energy structure respectively. The monomer orientation for the complex I, II, and III are in G'T form and the complex IV is in the GG form. The lowest energy complex is stabilized by two inter molecular H-bonds (OH···O_w and O_wH_w···N) between water and monomer while other complexes are having only a single intermolecular H-bond between the monomer and water.

The experimentally measured rotational spectrum of 2-MAE-H₂O was assigned to the lowest energy theoretical structure of the complex because of the excellent agreement between the

model and experimental rotational constants. The experimental rotational constants, A, B, and, C are within 0.9%, 1.7%, and 3.0% of the model values, respectively. As the predicted dipole moments of the complex I (see Table 7.2) suggested, the most intense lines were a-type. The b type and c-type lines were lower intensity resulting in 14 a-type transitions, 5 b-type transitions, and 4 c-type transitions. The ΔI_{rms} value ($\Delta I_{rms} = 6.4$ amu Å²) shows that the structure of complex I describes the 2-MAE-H₂O complex best.

The rotational spectra of two stable conformers of 2-MAE (G'T, and GG forms) were assigned in previous studies^{80,81}, but only the G'T monomer conformation of 2-MAE-H₂O complex was found experimentally in this study. This could be because of the steric hindrance from the methyl group of the GG monomer prevents formation of the new intermolecular H-bonding network that stabilizes complex I (monomer structures are shown in Figure 7.1). The ab initio calculations also indicate that the GG form of the water complex is higher in energy with respect to the lowest energy G'T form of the water complex by 24.71 kJ mol⁻¹ while the monomer study ab initio calculations predicted the GG form as the second lowest energy conformer at only 0.65 kJ mol⁻¹ higher than the lowest energy conformer⁸¹.

The main purpose of this study was to identify the structural changes of the 2-MAE monomer upon introducing a water molecule into the system. An intramolecular hydrogen bond from hydroxyl to amine nitrogen (OH…N) stabilizes the 2-MAE for each G'T and GG conformers. Introducing a water molecule to the system disrupts the intramolecular hydrogen bond and allows formation of a new network of intermolecular hydrogen bonds in the system; one hydrogen bond from hydroxyl to water and another one from water to amine nitrogen. Because of this new intermolecular hydrogen bonding network, the O - C - C - N dihedral angles(τ) opens up from -

57.7 ° (in the monomer) to -76.5 ° (in the water complex). The O…N separation also increases by 0.290 Å (shown in Figure 7.6).

The conformational changes observed in 2-MAE-H₂O complex are similar to the 2aminoethanol-water complex. In the 2-aminoethanol-water⁷⁹ complex the O - C - C - N dihedral angles(τ) increased by 18.0 ° from 57.0 ° in the monomer to 75.0 ° in the water complex as the τ increases by 18.8 °. The structural changes of glycidol monomer are smaller compared to 2aminoethanol and 2-MAE monomers in formation of their water complexes. In the glycidolwater³¹, the O – C – C – O dihedral angle(τ) increases by 9.1 ° from 40.8 ° to 49.9 °. The smaller increase in τ for glycidol results smaller change in the O···O separation upon water complexation (increases by 0.144 Å). The corresponding separation of O…N in 2-aminoethanol and 2-MAE have increased by 0.304 Å and 0.290 Å, respectively. Although glycidol has smaller structural changes compared to the 2-aminoethanol, and the 2-MAE upon water complexation, the new network of intermolecular hydrogen bonding network behave similarly for all three molecular complexes: OH…Ow and OwH…O are 1.92 Å and 1.88 Å in glycidol-water, and the corresponding OH…Ow and OwH…N intermolecular H-bonds are 1.89 Å and 1.88 Å in 2-aminoethanol-water and 1.90 Å and 1.84 Å in 2-MAE-H₂O, respectively. All these monomer molecules open their dihedral angles (τ) a measurable amount at formation of the water complex. These structural changes easily identified using rotation spectroscopy.



Figure 7.6. Comparison of the structural changes between MAE monomer and MAE-H₂O complex. O-C-C-N dihedral angle (τ) and C-C-N-C dihedral angle (χ).

7.5 Conclusion

Ab initio calculations (MP2/6-311++G(d,p)) were used to model the most likely complexes of 2-MAE-water complex. The rotational spectrum has been recorded for the 2-MAEwater complex in the 9-18 GHz frequency range. The experimental spectrum was found to be most consistent with the model complex I. Therefore, the experimental structure of the 2-MAE-water complex is assigned to the lowest energy model complex. Water complexation creates a new network of hydrogen bonding in the system: a hydrogen bond from water to methylamino nitrogen (O_w-H_w…N) and another hydrogen bond from hydroxyl to water (OH…O_w). Formation of this new network of intermolecular hydrogen bonds changes the monomer structure significantly, the OCCN dihedral angles(τ) was opened up in 18.8 ° and O…N distance increases by 0.290 Å. No methyl internal rotation tunneling splittings were observed in the 2-MAE-water rotational spectrum.

7.6 Optimized Molecular Coordinates for 2-MAE-Water Complex

Atom	Atom	a	b	c
Number				
1	С	0.295405	-1.176237	0.150635
2	С	-1.156897	-1.185490	-0.317948
3	0	-1.994364	-0.309083	0.405678
4	Н	-1.561169	-2.190361	-0.166473
5	Н	-1.189596	-0.974827	-1.399075
6	Ν	1.022427	0.012921	-0.318580
7	Н	0.793995	-2.102061	-0.184451
8	Н	0.304534	-1.166104	1.245584
9	Н	-1.722846	0.601878	0.212436
10	С	2.389501	0.059575	0.209735
11	Н	1.071822	-0.039671	-1.335083
12	Н	2.954698	-0.863200	0.011842
13	Н	2.346030	0.208564	1.292066
14	Н	2.925167	0.903014	-0.233042
15	0	-0.725022	2.175152	-0.139033
16	Н	0.043183	1.562548	-0.113771

Table 7.4. Optimized molecular structure for complex I of 2-MAE-water. The coordinates (Å) of water molecule is highlighted.

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17	Н	-0.535776	2.854134	0.512321

7.7 Assigned transition frequencies of 2-MAE-water complex.

J'	K'_a	<i>K</i> ' _{<i>c</i>}	F'	J''	$K_a^{\prime\prime}$	$K_c^{\prime\prime}$	<i>F''</i>	Obs	Obs-calc
								(MHz)	(MHz)
3	0	3	2	2	1	2	1	9935.472	0.001
			4				3	9935.512	0.001
			3				2	9935.709	0.000
3	1	3	2	2	1	2	1	10196.731	0.005
			4				3	10196.731	0.002
			3				2	10196.842	0.003
2	1	1	2	1	0	1	1	10215.755	0.002
			3				2	10215.871	0.002
3	0	3	3	2	0	2	2	10548.862	0.002
			4				3	10548.925	0.002
			2				1	10549.028	0.000
3	2	2	2	2	2	1	1	11461.315	0.001
			4				3	11461.590	0.000

Table 7.5. Assigned transition frequencies of 2-MAE-water complex

			3				2	11462.087	0.001
2	2	1	2	1	1	0	1	11641.816	0.004
			3				2	11642.178	0.004
2	2	0	2	1	1	0	1	11914.414	0.001
			3				2	11914.682	0.001
3	1	2	4	2	1	1	3	12363.004	0.003
			3				2	12363.109	0.003
3	2	1	2	2	2	0	1	12374.095	0.001
			4				3	12374.377	0.002
			3				2	12374.954	0.001
2	2	1	2	1	1	1	1	12386.607	0.007
			3				2	12386.769	0.007
2	2	0	1	1	1	1	1	12659.024	0.005
			2				1	12659.174	0.007
			3				2	12659.274	0.002
4	0	4	5	3	1	3	4	13301.199	0.002
			4				3	13301.263	0.002
4	1	4	5	3	1	3	4	13392.985	0.005
			4				3	13393.031	0.005
4	0	4	4	3	0	3	3	13562.379	0.001
			5				4	13562.407	0.001
4	1	4	4	3	0	3	3	13654.152	0.005
			5				4	13654.206	0.001
			3				2	13654.258	0.005
4	2	3	3	3	2	2	2	15062.963	0.004
			5				4	15063.020	0.005
			4				3	15063.202	0.001
4	3	2	3	3	3	1	2	15681.839	0.001
			5				4	15682.018	0.002
			4				3	15682.504	0.001
3	2	1	3	2	1	1	2	15903.184	0.000

			4				3	15903.364	0.001
			2				1	15903.496	0.001
4	1	3	4	3	1	2	3	16007.805	0.007
			5				4	16007.825	0.001
			3				2	16007.878	0.008
4	3	1	3	3	3	0	2	16045.378	0.002
			5				4	16045.568	0.002
			4				3	16046.107	0.000
5	1	5	6	4	1	4	5	16521.298	0.001
			4				3	16521.313	0.001
			5				4	16521.313	0.001
5	0	5	5	4	0	4	4	16584.214	0.002
			6				5	16584.214	0.002
4	2	2	3	3	2	1	2	16769.012	0.002
			5				4	16769.059	0.003
			4				3	16769.275	0.001

CHAPTER 8

CONCLUSION

Determining the molecular structure is important to understand the function of a molecule. High-resolution rotational spectroscopy is an invaluable tool to probe the precise 3-dimensional structures of molecules and molecular complexes in gas phase. It can be used to measure a molecule's bond lengths, bond angles, and dihedral angles precisely. The combination of computational chemistry and microwave spectroscopy technique was used to investigate the conformational properties of small biomolecules and molecular complexes. A cavity-based Fourier transform microwave (CB-FTMW) spectrometer was used to record (in the 8-22 GHz frequency range) all the spectra of the molecules and molecular complexes addressed here.

In this dissertation, we mainly focused on studying hindered methyl internal rotation (methyl torsion) in small molecular systems. The high resolution of the instrument allows us to record and assign the A- and E- tunneling components along with the nuclear quadrupole hyperfine components that result from the ¹⁴N nuclei. The methyl-internal-rotation barrier ranges from 393-

411 cm⁻¹ for the series of molecules (valine methyl ester, proline methyl ester, methyl heptanoate, and methyl octanoate) we investigated.

We recorded and assigned rotational spectra for the amino acid methyl esters valine methyl ester (ValOMe) and proline metyl ester (PrOMe). We observed two stable conformers of ValOMe and one conformer of PrOMe. The two lowest-energy structures of ValOMe have similar intramolecular H-bonding networks; the principal difference between these conformations is the orientation of the isopropyl side chain. The experimentally determined barriers to methyl internal rotation of ValOMe conformer I and conformer II are 401.64(19) cm⁻¹ and 409.74(16) cm⁻¹, respectively. The barrier height of the PrOMe was determined to be 393.54(9) cm⁻¹ from the spectroscopic fits.

We characterized the structure of two molecules of the family of methyl alkynoates, methyl heptanoate and methyl octanoate, with rotational spectroscopy and ab initio calculations. Spectra arising from two stable conformers, C_S and C_1 , were assigned for both molecules. The rotational transitions are split to A and E components due to internal rotation of the methyl group. The barrier to methyl internal rotation of methoxy methyl group is 399.4(10) cm⁻¹ for C_S and 411(4) cm⁻¹ for C_1 conformer of methyl heptanoate and for the methyl octanoate, 399.2(13) cm⁻¹ and 409.3(26) cm⁻¹ for C_S and C_1 conformers, respectively.

Rotational spectroscopy is also an ideal technique to identify changes in molecular structures upon formation of new hydrogen bonding networks. We investigated the hydrogen bonding motif and molecular structure of the 2-methylaminoethanol-water (2-MAE-water) complex. Using the spectroscopic data of the 2-MAE-water and the ab initio optimized structures, we have shown that the structure of the water complex is formed through a network of intermolecular hydrogen bonds: a hydrogen bond from water to methylamino nitrogen (O_w-

 $H_w \cdots N$) and another hydrogen bond from hydroxyl to water (OH $\cdots O_w$). Formation of this new network of intermolecular hydrogen bonds changes the monomer structure significantly; the OCCN dihedral angle(τ) increases by 18.8° and the O $\cdots N$ distance increases by 0.290 Å.

The rotational spectral data and computational calculation presented in this work provide the precise structural and methyl internal rotation barrier information of four molecules and a molecular complex. These intrinsic structural information will improve our understanding of molecular conformations in biological systems.

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