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# The effect of deuterium substitution in the amino group on the internal-rotation barrier of acetamide

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ABSTRACT

Peptide molecules XCO–NYY′ are characterized by low potential barrier $V_3$ to internal rotation of a methyl group substituted for *X* and/or *Y*. A most conspicuous example is acetamide, for which $V_3$ was previously reported to be 25.043857(19) $cm^{-1}$ [8]. The present study intended to clarify why $V_3$ is so low in acetamide, by examining the effect of the out-of-plane bending or inversion of the amino group on the molecular structure through deuterium substitution for amino hydrogens. The potential barrier $V_3$ in acetamide was found to decrease by 2.630, 2.986, and 5.532 $cm^{-1}$, when H′s at *cis*, *trans*, and both positions in the amino group were replaced by deuterium atoms, respectively. The reduction was proportional to the effective mass of the out-of-plane bending mode of the amino group (hereafter referred to as the amino inversion), which was in turn ascribed to the change in electronic resonance character of the peptide linkage. The amino inversion is coupled with the $CH_3$ internal rotation, producing an interaction term proportional to $\tau \sin 3\alpha$, where $\tau$ and $\alpha$ denote the amino inversion and methyl internal rotation angles, respectively. This coupling term, when the inversion is treated by second-order perturbation, yields a $V_6$ term in the internal-rotation potential function of the methyl group, in agreement with the finding of Ilyushin et al. [8], who derived an unusually large $V_6$ term of $-10.044874(73)$ $cm^{-1}$. It is quite interesting that even a small perturbation such as deuterium substitution causes a substantial change in electronic structure of the peptide linkage.



## 1. Introduction

Peptide molecules defined by the chemical formula: XCO–NYY′ play important roles in many areas, in particular in biological systems such as proteins. Kawashima and his collaborators have carried out a systematic study on the molecular structure of peptide molecules, by paying particular attention to the internal rotation of methyl groups substituted at the *X* and *Y* (*trans* to *X* with respect to the central C—N bond), and sometimes at the *Y*′ (*cis* to *X*) positions, in order to elucidate the essential feature of the dynamical structure of the peptide systems, as summarized in [1]. In most peptide molecules thus far examined, the potential barrier $V_3$ to internal rotation of $CH_3$ at *X* and *Y* lies between 50 and 100 $cm^{-1}$, *i.e.* in the range that is much lower than that found for $CH_3$ in organic molecules of similar size. These low potential barriers are of considerable significance for the conformations which prevail in large and often biologically important molecules with peptide linkages as the main skeletal backbone units. Among simple peptide molecules, acetamide stands out by its unusually low $V_3$ and thus we anticipate that it may provide us with a clue to clarify the structural and dynamical characteristics inherent in the peptide molecular systems.

Kojima, Tsunekawa, and their collaborators initiated the microwave study on acetamide in 1985 [2,3] and continued their investigation by extending the frequency range further up to 200 GHz [4]. Heineking and Dreizler [5] focused attention to the hyperfine structure due to nitrogen nuclear quadrupole coupling. Suenram et al. [6] reinvestigated acetamide by introducing a new method: Fourier transform microwave spectroscopy (FTMW) and carried out a global analysis using the $\rho$-axis method (RAM) [7], where they included all the data reported in [3,5] in addition to their own FT spectra. More recently Ilyushin et al. [8,9] further extended the observation not only to pure rotational transitions in the ground vibrational state, but also to those in excited states of $CH_3$ internal rotation and to torsion (*i.e.* internal rotation)-rotation transitions, thereby covering the spectral data on the torsional states with the torsional quantum number up to $v = 2$ and determined both the $V_3$ and $V_6$ internal-rotation potential constants. No microwave studies were, however, performed on isotopic species, and the structural parameters were provided so far only by an electron diffraction study of Kitano and Kuchitsu [10].

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Among the isotopic species of acetamide, those with deuterium atoms in the amino group are of particular interest. The main skeleton of the heavy atoms of this molecule is essentially planar, and thus the two vibrational modes of interest: the methyl internal rotation and the amino out-of-plane wagging (or inversion) both belong to same $A''$ symmetry species and may thus be expected to couple with each other to a considerable extent. In this respect, it is interesting and important to point out that, in the simplest peptide molecule formamide, the amino wagging is governed by a potential function with a large quartic character [11]. In the present work focus was directed mainly to the isotopic species of acetamide with deuterium atoms substituted in the amino group, in order to obtain valuable information on the dynamical structure of this molecule as a whole from its FTMW spectra.

## 2. Experimental

There are three amino-deuterated species for acetamide: the species with a deuterium in the *trans* (*Y*) and *cis* (*Y'*) positions of the amino group (see Fig. 1), which are referred to as ND-*trans* and ND-*cis*, respectively, and the one with both amino hydrogens replaced by deuterium atoms, called ND2, while the normal species is named NH2 for the sake of consistency. The deuterated species of acetamide were generated by mixing the normal species with $CD_3OD$, before introducing the sample in the cavity of a FTMW spectrometer installed at Kanagawa Institute of Technology. Samples of normal as well as deuterated species of acetamide were introduced in an absorption cell of the FTMW spectrometer, by using Ar as a carrier gas at the backing pressure of approximately 3 atm, through a pulsed valve maintained at 70–110 °C. We scanned the frequency region from 4 to 35 GHz, while integrating the signals 20–100 times to get a good signal-to-noise ratio. The details of the spectrometer employed are described in detail in [12].

## 3. Observed spectra and analysis

Because of an extremely low barrier to $CH_3$ internal rotation in acetamide, the analysis of its rotational spectra is by no means routine. This situation is clearly demonstrated in Table 1, which compares the rotational constants and $V_3$ reported in the past [5,4,6,8] among others; the rotational constants of [4,6,8] were originally determined by the RAM, but were converted for comparison to those defined in terms of the principal axes of inertia, as done in the framework of the principal axis method (PAM), by using the following relations:

$$A + B = A_R + B_R, \tag{1a}$$

$$A - B = [(A_R - B_R)^2 + 4D^2]^{1/2}, \tag{1b}$$

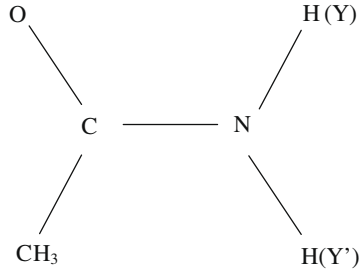


**Fig. 1.** Acetamide molecule. According to a convention widely accepted, the *Y* and *Y'* positions are called *trans* and *cis*, respectively.

**Table 1**
Rotational constants (MHz) and the potential barrier $V_3$ ($cm^{-1}$) to $CH_3$ internal rotation of acetamide.

| Refs. | [5] | [4][a] | [6][a] | [8][a] | Present[a] |
|---|---|---|---|---|---|
| *A* | 10839.43 | 10354.595[b] | 11524.16 | 10803.824 | 10824.129 |
| *B* | 9285.20 | 9260.610[b] | 9293.43 | 9302.730 | 9284.797 |
| *C* | 5156.15 | 5175.206 | 5086.28 | 5156.779 | 5156.105 |
| $V_3$ | 24.932 | 24.3906 | 24.34 | 25.043857 | 25.467840 |
| $V_6$ | [0.0][c] | [0.0][c] | [0.0][c] | −10.044874 | [−10.044874][c] |
| Δ[d] | 3.03777 | 5.72630 | −1.12711 | 3.10082 | 3.10519 |

[a] The rotational constants derived by RAM are converted to those in the principal inertial axis system.
[b] According to a private communication from H. Odashima, the *A* constant listed in [4] corresponds to $A + F\rho^2$. This correction $F\rho^2$ is included in calculating *A* and *B* in this Table.
[c] Assumed.
[d] $I_{aa} + I_{bb} - I_{cc}$ in uÅ$^2$.

where $A_R$ and $B_R$ denote the *A* and *B* rotational constants defined by the RAM formulation and *D* is an off-diagonal constant, whereas *C* remains unchanged by the RAM transformation, because the molecule is assumed to be planar. It is striking to see how different the four published sets of data [5,4,6,8] are from each other. A part of the discrepancies probably arises from too many parameters being included in the fits, making the physical meaning of each parameter somewhat obscure, because of correlation among them. One way of confirming how reliable each data set is, is to calculate $\Delta = I_{aa} + I_{bb} - I_{cc}$ and to compare it with the moment of inertia of the methyl top about its internal-rotation axis. The electron diffraction data [10] lead to 3.3815 uÅ$^2$. The values of [4] and [6] deviate much from it, whereas the Δ's of [5], [8] and [present] are close to, only slightly smaller than the electron diffraction value, which is reasonable, because intramolecular vibrations tend to reduce the Δ value by a small amount known as the contribution of the pseudo-inertial defect. The *C* constant calculated from the structural data of [10], 5105.63 MHz, may be scaled against an average of the three *C* values [5,8,present]: 5156.345 MHz to calibrate the *A* and *B* constants (calculated to be 10666.96 and 9190.68 MHz) to 10772.01 and 9281.97 MHz, respectively, both of which are smaller than the respective values reported in [5,8,present], suggesting a slight modification required for the structural parameters of [10] to reproduce the microwave data.

In the present study, we were mainly interested in effect of deuterium substitution in the amino group on the internal-rotation barrier of acetamide. In estimating such shifts, we certainly needed a reference, the data on the normal species, and thus we repeated the analysis of the spectra of the normal species before we examined those of the deuterated species. In order to avoid excessive correlations, we limited the number of parameters to as small as possible, while focusing attention to the differences in molecular parameters, mainly those in $V_3$ barrier height.

For the normal species, we measured by FT the frequencies of 23 and 24 rotational transitions with *J* up to 6 and 4 for the *A* and *E* internal-rotation states, respectively, as listed in Table 2, where only the strongest $\Delta F = \Delta J$ among nitrogen hyperfine components are listed (a complete list is given in Supplementary Data as Table S-1). We labeled the *E* internal-rotation states by a symmetric-top like notation: *J*, *k*, with assigning *k* the numbers: 0, 1, −1, 2, −2,..., according to the increasing order of energy. This labeling scheme of the eigenstates may little reflect physical nature of each level, but is simple and unique in most cases, and is thus more versatile than those adopted in [4,6,8,9]. We employed a computer program described in detail in [13]. The main parameters included 3 rotational and 5 centrifugal distortion constants for each of the *A* and *E* internal-rotation states, the *D* constant, the internal-rotation potential barrier $V_3$ in addition to two minor centrifugal correction terms, $\Delta_{EKJ}$ and $\Delta_{EKX}$, thus the total number of parameters was 20 (except for 3 nitrogen hyperfine constants)