

# LETTERS TO THE EDITOR

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## COMMUNICATIONS

### Infrared laser spectroscopy of uracil in a pulsed slit jet

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A new high-temperature pulsed slit jet source has enabled the first observation of a rotationally resolved vibrational spectrum of a nucleotide base. The spectrum, centered at  $1703.888\text{ cm}^{-1}$ , has been assigned to the fundamental out-of-phase mixed carbonyl stretching vibration of the diketo tautomer of uracil, clarifying an ambiguous assignment from low-resolution studies. © 1995 American Institute of Physics.

#### INTRODUCTION

The importance of the five natural nucleotide bases in determining the structure and function of both RNA and DNA is unquestionable. The double helix structure<sup>1</sup> results largely from the specificity of the hydrogen bonds between the base pairs. Detailed information on this hydrogen bonding (geometries, potential-energy surfaces) can be extracted from high-resolution spectra of the base pair dimers.<sup>2</sup> However, before such investigations can be undertaken, it is necessary to characterize the nucleotide base monomers in detail. To address this, previous experiments have led to the observation of both electronic<sup>3</sup> and rotational<sup>4–7</sup> spectra of several nucleotide bases, exploiting supersonic pinhole expansions to minimize an otherwise prohibitive spectral congestion. It would be very desirable to measure vibration–rotation spectra of these species because of the well-recognized high chemical information content of infrared spectra. Furthermore, the sensitivity and resolution advantages afforded by a two-dimensional slit jet for such measurements are well known.<sup>8,9</sup> Accordingly, we have developed a heated pulsed slit jet source for producing supersonic expansions of nonvolatile biological molecules. In this Letter we describe the application of this technology for the first measurement of a vibration–rotation spectrum of a nucleotide base.

Uracil, shown in Fig. 1, was chosen for the present study because it is the simplest and most thermally stable of the five nucleotide bases. As is characteristic of all the bases, uracil is nonvolatile, having a vapor pressure of only 75 mTorr at 210 °C.<sup>10</sup> Consequently, the majority of previous infrared spectroscopic investigations have studied uracil in solution,<sup>11</sup> trapped in low-temperature Ar or N<sub>2</sub> matrices,<sup>12–16</sup> or as a polycrystalline sample.<sup>17</sup> None of these studies were able to resolve rotational structure. *Ab initio* Hartree–Fock SCF calculations have been employed to interpret the matrix isolation spectra.<sup>16,18–20</sup> However, controversy exists concerning the assignment of the vibrational modes, in particular, those involving the carbonyl stretching motions. This has arisen, at least in part, because of spectral congestion in the 1600–1800  $\text{cm}^{-1}$  region, and as a result of

the frequency shifts induced by a matrix. Nevertheless, the vibrational frequencies obtained from the matrix isolation studies have proved valuable for locating vibrational bands in the present study. Previous experiments on uracil in the vapor phase include direct absorption infrared spectroscopy using a heated gas cell<sup>21</sup> and an electron-diffraction study.<sup>22</sup> The elevated temperatures required in the former experiment produced linewidths in excess of  $25\text{ cm}^{-1}$ , hence obscuring any rotational structure. The microwave spectrum of uracil was recorded by Brown *et al.*<sup>4</sup> They assigned 65 rotational transitions, and from the resulting ground-state parameters, determined that the diketo tautomer of uracil was the dominant vapor phase species. This was in agreement with a number of theoretical studies of the relative stabilities of the six possible tautomers.<sup>23–25</sup>

Despite the considerable literature on uracil, no high-resolution infrared spectroscopic measurements have been reported. We describe here the first observation of a rotationally resolved vibrational spectrum of uracil in the gas phase, which has been assigned to the out-of-phase  $\nu_6(\text{C}_2=\text{O}, \text{C}_4=\text{O})$  stretching vibration.

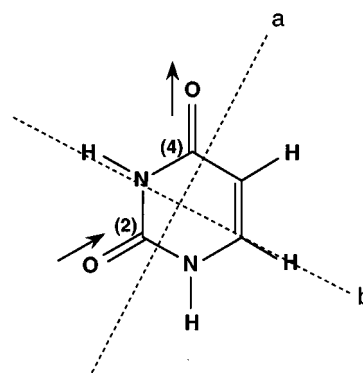


FIG. 1. Molecular structure of the diketo tautomer of the RNA base uracil with the *a*- and *b*-inertial axes indicated; the numbers refer to ring positions. The two arrows represent the principle atomic motions contributing to the observed out-of-phase stretching vibration.

## EXPERIMENT

A direct absorption infrared diode laser experiment similar to the one used in the current study has been described previously,<sup>26</sup> and hence only a brief summary will be presented here. A detailed description of the design and operation of the high-temperature pulsed slit jet source will be reported elsewhere.<sup>27</sup> The infrared radiation was obtained from a helium-cooled diode laser spectrometer (Spectra-Physics) using  $\text{Pb}_{1-x}\text{Se}_x$  diodes (Laser Analytics). A single diode gave almost continuous frequency coverage from 1701 to 1706  $\text{cm}^{-1}$ .

A free jet expansion of uracil was produced using a high-temperature pulsed slit molecular beam source. The slit, with dimensions 10 cm by 100  $\mu\text{m}$ , is sealed by a Viton cord set in an aluminum poppet. The poppet, in turn, is connected to three high-temperature solenoid valves (General Valve series 9) which allow the source to be pulsed up to 80 Hz. However, for the work reported here a repetition rate of 35 Hz was employed. Uracil (minimum 99% pure, Sigma Chemical Company) is contained within a detachable sample chamber located on the bottom of the source. In order to produce a sufficient vapor pressure of uracil, the entire gold coated copper source is heated to approximately 210 °C by two 150 W heaters located on the top and bottom faces of the source. The top heater is set at a temperature approximately 10 °C higher than the bottom heater to ensure that no condensation of uracil vapor occurs in either the main source body or the slit. Three type-*J* thermocouples allow the temperature of the source to be monitored. Vaporized uracil diffuses from the sample chamber up into the main housing via an array of holes located just below the poppet. After mixing with helium carrier gas at a backing pressure of 3 atm, the uracil expands into a vacuum chamber maintained at a pressure of 250 mTorr by a 1000  $\text{s}^{-1}$  roots pump. The helium is preheated before entering the source to help reduce condensation of the uracil vapor.

The uracil expansion is intersected between 0.5 and 2 cm downstream of the slit by the infrared laser beam, which is multipassed about 22 times using a Herriott cell configuration;<sup>28</sup> this gives an effective path length of 220 cm. Transient absorption signals measured with a HgCdTe photoconductive detector (Santa Barbara Research Associates) are amplified, filtered, and then averaged by a pair of model SR250 gated boxcar integrators (Stanford Research Systems); the resulting signal is recorded by a 486-DX PC, which also controls the scanning of the laser. The averaged time profile of the detector output is observed on a Tektronix TDS 320 digital oscilloscope, allowing accurate placement of the two boxcar gates. The timing of the entire experiment is controlled by a model DG535 pulse generator (SRS). Frequency calibration of the observed transitions requires the simultaneous scanning of both an OCS or  $\text{H}_2\text{O}$  reference gas and the fringe spacing of a vacuum-spaced étalon.

## RESULTS AND DISCUSSION

A total of 446 peaks have been observed between 1701.5 and 1706.1  $\text{cm}^{-1}$ . Continuous coverage of this 4.6  $\text{cm}^{-1}$  region was achieved except for a small 0.27  $\text{cm}^{-1}$  gap, cen-

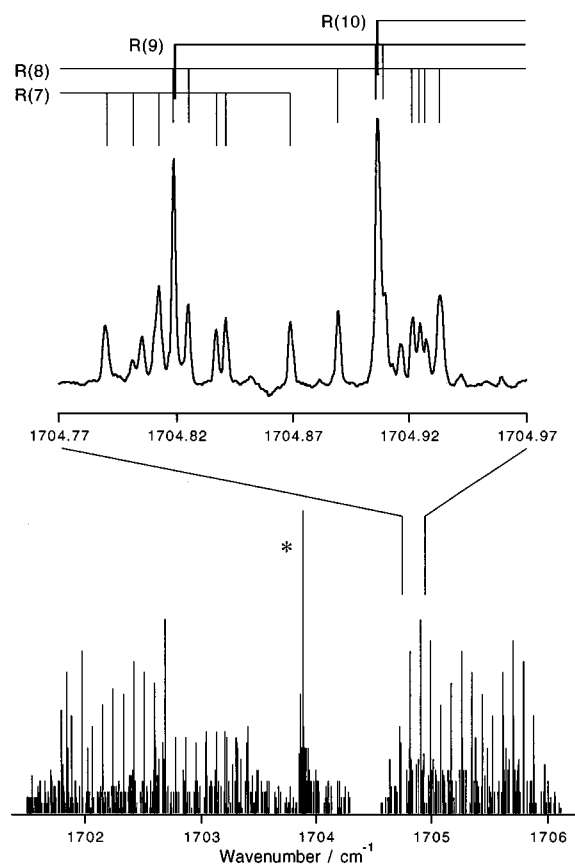


FIG. 2. The lower spectrum shows a stick diagram of all 446 observed peaks with their relative intensities, except for the peak marked with an asterisk which is depicted at one-tenth of its true magnitude. The spectrum has been assigned as an *a*-type band of the out-of-phase  $\nu_6(\text{C}_2=\text{O}, \text{C}_4=\text{O})$  stretching vibration. Presented above is an actual experimental spectrum corresponding to a 0.2  $\text{cm}^{-1}$  section of the *R* branch. The peaks have Doppler broadened linewidths of 75 MHz, and have been assigned as indicated. An upper limit for the rotational temperature of the uracil is estimated to be 20 K.

tered at 1704.43  $\text{cm}^{-1}$ , caused by the loss of laser power due to a strong water absorption. A stick spectrum of all observed peaks, with their relative intensities, is shown in the lower half of Fig. 2. The jagged intensity profiles of the most intense progressions result predominantly from a fluctuation of the laser power with frequency. The spectrum is dominated by a single intense peak at 1703.888  $\text{cm}^{-1}$ , which in the stick spectrum is depicted at one-tenth of its true intensity. This peak has a linewidth (FWHM) of 100 MHz and a signal-to-noise ratio in excess of 1000:1, corresponding to 20% absorption of the laser radiation. However, the majority of peaks have signal-to-noise ratios up to 100:1, and Doppler broadened linewidths of 75 MHz. The most significant source of noise is the vibration of the diode laser cold head. An actual experimental spectrum is shown in the upper half of Fig. 2; the peaks have been calibrated to a frequency accuracy of 40 MHz and assigned to transitions *R*(7) through to *R*(10).

Using a nonlinear least-squares method the spectrum was fit to an *s*-reduced Watson Hamiltonian,<sup>29</sup> and has been assigned as an *a*-type band of an asymmetric rotor.<sup>30</sup> The

TABLE I. Optimized spectroscopic parameters for the ground and vibrationally excited states of uracil, including results from both the current work<sup>a</sup> and the previous microwave study (see Ref. 4). Numbers in parentheses are one standard deviation, and the standard deviation of the total fit is 0.894.

Constants	Microwave study	This work	
	Ground state	Ground state	Excited state
$A/\text{MHz}$	3883.87825 (110)	3883.87731 (80)	3886.63 (71)
$B/\text{MHz}$	2023.73267 (101)	2023.73252 (85)	2023.00 (19)
$C/\text{MHz}$	1330.92380 (60)	1330.92369 (50)	1330.08 (6)
$D_J/\text{kHz}$	0.06029 (77)	0.06010 (64)	-1.82 (50)
$D_{JK}/\text{kHz}$	0.1047 (14)	0.1048 (11)	-21.6 (70)
$D_K/\text{kHz}$	0.4724 (43)	0.4701 (34)	138 (18)
$d_1/\text{kHz}$	-0.02738 (28)	0.02736 (24)	-1.33 (27)
$d_2/\text{kHz}$	-0.006532 (94)	-0.00656 (8)	1.16 (13)
Vibrational energy/ $\text{cm}^{-1}$	0.0	0.0	1703.88822 (16)

<sup>a</sup>Transition frequencies are available from the authors on request.

total number of transitions in the fit included 65 transitions from the microwave study on the ground state<sup>4</sup> and 380 rovibrational transitions from the current work; the transitions were properly weighted according to their frequency uncertainty. The resulting rms error of the residuals was 29.0 MHz, well within our estimated frequency accuracy. Due to the high line density in the infrared spectrum (typically 100 lines per  $\text{cm}^{-1}$ ) many of the transitions overlap with one another. Consequently, the 380 rovibrational transitions included in the fit, with  $J$  ranging from 0 to 23, account for only 232 distinct peaks in our data set. Many remaining weak unassigned peaks result from transitions between levels characterized by high  $J$  and  $K_a$ . The rotational temperature of the uracil cannot be determined accurately because of the frequency dependence of the laser power. However, from the overall profile of the observed spectrum we have estimated an upper limit of 20 K. The spectroscopic parameters derived from both the present fit and from the previous microwave study are shown in Table I. The ground-state parameters derived from the two fits are in excellent agreement, allowing us to conclude that the diketo tautomer of uracil has been observed in the heated slit jet expansion. For the excited state, the centrifugal distortion parameters appear unrealistically large, in particular, the value of  $D_K$ . These anomalous values almost certainly result from perturbations to the excited state. It is possible that some of the unassigned peaks in the spectrum originate from other tautomers of uracil in the expansion. However, because the diketo species can account for all but the weakest peaks in the observed spectrum we can conclude that it is the dominant tautomer.

The 1600–1800  $\text{cm}^{-1}$  region of the uracil spectrum is known to be very complex. In addition to the three fundamental vibrational modes resulting from C=O and C=C stretches, matrix isolation studies<sup>12–16</sup> have demonstrated the presence of up to eight combination bands. These combination bands are unusually intense due to Fermi resonance with the fundamental modes. We have assigned the observed band at 1703.888  $\text{cm}^{-1}$  to the fundamental  $\nu_6$  mode, arising predominantly from an out-of-phase mixed carbonyl stretching vibration. This assignment is based upon a number of argu-

ments. First, the considerable strength of the observed spectrum is characteristic of the  $\nu_6$  carbonyl vibration, which has a calculated intensity of 1226  $\text{km mol}^{-1}$ .<sup>20</sup> Second, the predominant projection of the change in dipole moment during this out-of-phase vibration lies along the  $a$ -inertial axis, as shown in Fig. 1. Consequently, one would expect the vibrational band to be  $a$  type, as observed. This assignment is consistent with the matrix isolation studies, all of which observed an intense feature at 1706 or 1707  $\text{cm}^{-1}$  in an Ar matrix. Two of these studies<sup>13,15</sup> specifically assigned this intense peak to the  $\nu_6$  mode. Furthermore, these papers report a weaker combination band centered at 1698 or 1699  $\text{cm}^{-1}$ , which we have also observed. More unassigned peaks in our data set can be accounted for by the overlap of this combination band with the fundamental  $\nu_6$  band.

Following the successful characterization of a nucleotide base in the vapor phase, attempts are now underway to study other bases, their corresponding nucleosides, and biologically important clusters. We are currently searching for intramolecular vibrational modes in both the uracil dimer and uracil–water complex. Although the matrix isolation spectra of these clusters<sup>31,32</sup> guide our search, observations at mid-infrared frequencies are often complicated by spectral congestion. In the future, our tunable far-infrared laser spectrometer will be employed to complement the diode laser experiment, as it has recently been shown that detailed information on the hydrogen bonding within clusters can be extracted from high-resolution measurements of intermolecular vibrations.<sup>33</sup>

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