# Microwave Spectrum of the Major Gas-phase Tautomer of Thymine

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The microwave spectra of the nucleic acid base thymine and of two of its mono-deuteriated species have been analysed indicating that the diketo tautomer is the most abundant form in the gas phase.

As a continuation of previous studies of biologically significant molecules, which led to our reporting on the microwave spectra of urea,[1] glycine,[2] and uracil,[3] we now present data on the microwave spectrum of thymine, another of the nucleic acid bases. Preliminary studies of the vaporisation of thymine revealed that vaporisation without decomposition was possible under carefully controlled conditions. With our recently developed Stark-modulated spectrometer,[4] microwave measurements on a seeded supersonic beam of thymine in argon expanded through a 550 μm diameter nozzle proved successful. The microwave spectrum of the heterocycle has been observed at adequate S/N in the vicinity of 60 GHz. The spectroscopic parameters, derived from 59 assigned lines are presented in Table 1.

The value of the inertial defect of thymine is that expected for an essentially planar cyclic molecule bearing a single methyl group, although slight deviations from planarity would go undetected. Furthermore the rotational constants agree to within better than 1% with those expected for the diketo tautomer (Figure 1, $T_1$), the tautomer predicted to be of lowest energy, although the constants for two other tautomers, predicted to be the two of next lowest energies ($T_2$ and $T_3$), are also compatible with the observed *A*, *B* and *C* (Table 2). However, the nature of the tautomer is settled unambiguously by analysis of the rotational constants determined for the two mono-deuterio species that we have also studied. These were prepared from the common isotopomer by simple exchange with heavy water in a Soxhlet extractor over 24 h and so the deuterium must be attached either to one of the nitrogens or one of the oxygens. By use of Kraitchman's equations the co-ordinates of the deuterium in the principal inertial axes system can be derived in each case (Table 1). Comparison with the estimated co-ordinates for each of the three plausible tautomers (Table 2) clearly indicates that it is tautomer $T_1$ that is being observed.

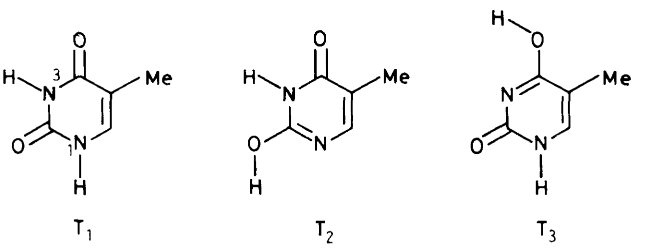

**Figure 1.** The three lowest energy tautomers of thymine.

The pattern of $^{14}N$ quadrupole hyperfine structure of spectral lines provides an independent, unambiguous method of identifying the tautomeric forms of molecules such as thymine where nitrogen atoms are involved in the tautomerism. The distinction is easy because pyrrolic nitrogens have a negative coupling constant (and with the largest magnitude) in the *c*-direction, *i.e.*, perpendicular to the heterocyclic ring. Typically $\chi_{cc}$ is in the vicinity of -3 MHz for pyrrolic nitrogens, $\chi_{aa}$ and $\chi_{bb}$ both being positive.[5,6] In sharp contrast, pyridinic nitrogens have positive values of $\chi_{cc}$, the component of largest

**Table 1.** Derived spectroscopic and geometric parameters for thymine.[a]

| | Thymine | N(1)-D-thymine | N(3)-D-thymine |
|---|---|---|---|
| *A* | 3 201 208.8(20) | 3 125 986.7(20) | 3 124 924.4(19) |
| *B* | 1 404 811.0(27) | 1 392 060.1(36) | 1 401 168.1(37) |
| *C* | 982 609.5(24) | 969 262.4(25) | 973 554.4(23) |
| $D_J$ | 0.0301(18) | 0.0347(19) | 0.0257(19) |
| $D_{JK}$ | 0.09768(70) | 0.0970(14) | 0.0963(13) |
| $D_K$ | 0.3908(86) | 0.3420(98) | 0.3563(89) |
| $d_1$ | −0.01078(62) | −0.01223(79) | −0.00968(93) |
| $d_2$ | −0.00220(20) | −0.00250(22) | −0.00192(25) |
| $\Delta/\mu Å^2$ | −3.297 | −3.308 | −3.302 |
| $\lvert a\rvert$/pm | | 180 | 96 |
| $\lvert b\rvert$/pm | | 197 | 197 |
| $\lvert c\rvert$/pm | | 8 | 5 |
| No. of lines[b] | 59 | 47 | 51 |

[a] In kHz except for Δ and *a*, *b* and *c*. [b] Transition frequencies are available from the authors upon request.

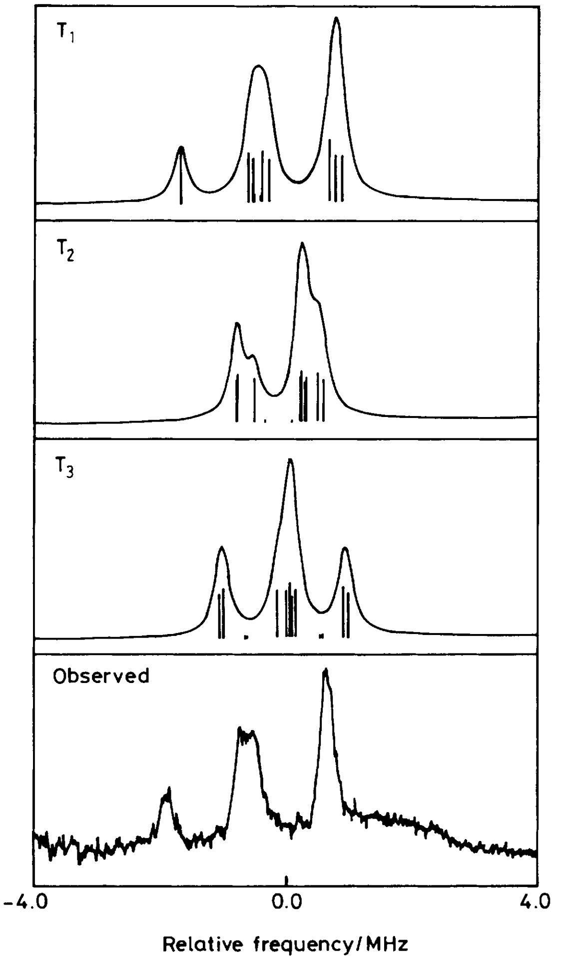

**Figure 2.** Observed and predicted hyperfine structure of the $14_{4,10}$—$13_{3,11}$ transition for the three lowest energy tautomers of thymine.

magnitude in this case being $\chi_{aa}$ or $\chi_{bb}$, the negative component being the one lying nearest in direction to the direction in which the nitrogen lone-pair is located.[6] While it has not proved feasible to analyse numerically the incompletely resolved multiplets we have observed in the thymine spectrum, the particular tautomer can easily be identified by computing the coupling constants *via ab initio* molecular-orbital calculations using relatively modest basis sets[7] and then predicting the expected multiplet shapes using a suitable code for the two-nuclei quadrupole hyperfine coupling.† Field gradients were calculated for each tautomer using a 3-21G basis with the GAUSSIAN 82 suite of programs.[8] Figure 2 shows the results for the $14_{4,10}$—$13_{3,11}$ multiplet, from which it is immediately apparent that the observed multiplet comes from tautomer $T_1$.

We have not detected any strong lines that could be assigned to other tautomeric forms, implying that the diketo form is the most stable tautomer in the gas phase. The sensitivity of our search for lines of the other plausible tautomers was such that, taken together with theoretical estimates of dipole moment components, we would have just detected other tautomers if present in more than about 10% abundance. This is in agreement with various theoretical studies of the relative stabilities of the tautomers,[9] and the tautomer observed in the crystal.[10] The inference that a small fraction of the keto-enol tautomer exists in the gas phase, based on the detection of two band systems in a fluorescence study,[11] has been shown to be incorrect.[12]

**Table 2.** Predicted parameters[a] for the three lowest energy tautomers of thymine.

| | $T_1$ | $T_2$ | $T_3$ |
|---|---|---|---|
| *A*/MHz | 3256 | 3293 | 3227 |
| *B*/MHz | 1414 | 1404 | 1426 |
| *C*/MHz | 992 | 990 | 995 |
| rel. *E*/kJ mol$^{-1}$ | 0 | 71.0 | 86.7 |

Predicted H-co-ordinates in the principal inertial axes sytems.

| | N(1)–H | O–H | N(1)–H |
|---|---|---|---|
| *a*/pm | −178.6 | −336.5 | −184.0 |
| *b*/pm | −197.1 | −44.6 | −192.6 |
| | N(3)–H | N(3)–H | O–H |
| *a*/pm | −95.6 | −98.5 | 113.5 |
| *b*/pm | 195.5 | 192.4 | 258.7 |

Predicted $^{14}$N coupling constants (MHz).

| | | | |
|---|---|---|---|
| $\chi_{aa}$[N(1)] | 1.81 | 0.05 | 1.65 |
| $\chi_{bb}$[N(1)] | 1.78 | −0.6 | 1.45 |
| $\chi_{cc}$[N(1)] | −3.59 | 0.55 | −3.10 |
| $\chi_{aa}$[N(3)] | 1.78 | 1.47 | 1.77 |
| $\chi_{bb}$[N(3)] | 1.53 | 1.51 | −2.63 |
| $\chi_{cc}$[N(3)] | −3.31 | −2.98 | 0.86 |

[a] Derived from the reoptimised *ab initio* 3-21G geometries assuming planarity of heavy atoms (Gaussian 82[8]).

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† A FORTRAN program QUAD 2 is available from the authors on request.