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Gas-Phase Tautomeric Equilibrium of 4-Hydroxypyrimidine with Its Ketonic Forms: A Free Jet Millimeterwave Spectroscopy Study

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Abstract: 4-Hydroxypyrimidine (4HP) has two conformational forms (the hydroxyl hydrogen cis or trans with respect to the adjacent nitrogen), which are in tautomeric equilibrium with two ketonic forms, 4-pyrimidinone (4PO) and 6-pyrimidinone (6PO). We have investigated the free jet absorption millimeterwave spectrum of this system, assigning the rotational spectra of 4HPcis and 4PO; the latter species is more stable by 2.0(9) kJ/mol. No lines corresponding to the trans isomer of 4-hydroxypyridine and to 6PO have been observed.

Introduction

The nucleobases cytosine, thymine, and uracil are pyrimidine derivatives. They pair with their complementary purines (guanine and adenine) through hydrogen bonding to form DNA and RNA chains. These nucleobases are, in the gas phase, complicated chemical systems where several tautomers and conformers are in equilibrium with each other. Four, one, and one species have been detected for cytosine,[1−4] thymine,[5−7] and uracil,[8−10] respectively, in the gas phase.

4-Hydroxypyrimidine presents the same kind of tautomeric and conformational possibilities as well, but it is slightly simpler with respect to the three bases mentioned above; the investigation of its equilibrium can help in understanding the nature of the forces which govern it. Such a kind of equilibrium takes place through an intramolecular hydrogen transfer. Hydroxypyridines, still simpler systems, are in tautomeric equilibrium with their ketonic forms.[11−14] In particular, 2-pyridinone/2-hydroxypyridine represents a prototype system for the investigation of tautomerism in aromatic ring basis, and its structural problem has been discussed already by Meyer in 1905.[15] After that, a rich literature has grown and a variety of chemical and physical methods have been brought to bear on deciding upon the structure, or position of equilibrium, of this tautomeric system.[11,12,16−20] Among them, myriads of theoretical calculations on this theme, often in conflict with each other, are available.[21] We believe that the best value of the relative tautomeric energy is given in our rotational spectroscopy report.[11]

2-Pyridinone/2-hydroxypyridine (2PO/2HP) and 4-hydroxypyrimidine and its keto forms (4HP/4PO/6PO) and uracil contain, at least in one tautomeric form, the peptidic group (−CO−NH), which is very interesting in biochemistry. The number of CO and NH groups inserted in the heterocycle strongly affects the direction of the keto-enolic equilibrium. In Chart 1, we show, in order of increasing complexity, the tautomeric species plausible for the three systems mentioned above. The trends of the equilibria, indicated qualitatively by

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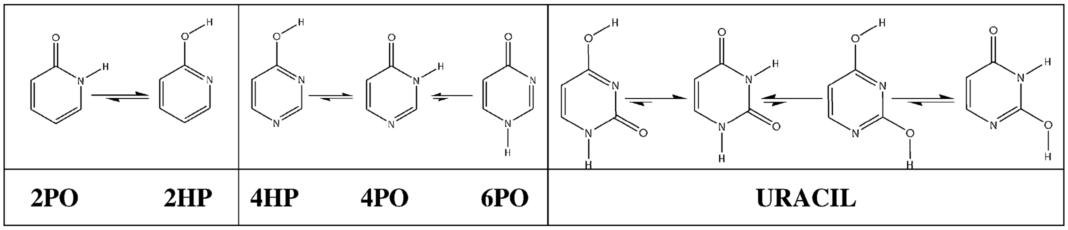
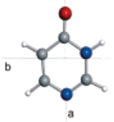
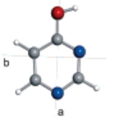
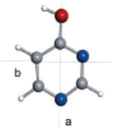
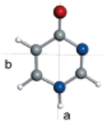
Chart 1. Tautomeric Equilibria in 2PO/2HP, 4HP/4PO/6PO, and Uracil[a]



[a] The lengths of the arrows represent, qualitatively, how much the equilibrium is shifted toward a certain species.

Table 1. Calculated B3LYP/6-31G** Spectroscopic Constants and Energies of the Four Stable Species of 4-Hydroxypyrimidine and Its Keto Forms[c]

	4PO	4HP		6PO
		cis	trans	
				
A / MHz	5974 / 5997	6164 / 6158	6185	5838
B / MHz	2791 / 2777	2782 / 2771	2762	2826
C / MHz	1902 / 1898	1917 / 1911	1909	1904
μ_a / D	-1.6 / -1.6	1.2 / 1.1	1.4	-7.4
μ_b / D	-1.5 / -1.4	0.5 / 0.7	3.3	1.7
E / kJ mol^{-1}	0[a] / 4.2	5.2 / 0[b]	27.8	42.3

[a] Absolute energy −339.569650 E_h. [b] Absolute energy −338.735372 E_h. [c] MP2/6-311++G** are given (to the right of the / sign) for the two most stable forms.

Table 2. Spectroscopic Constants of the Most Abundant and Monodeuterated Isotopologues of the Two Observed Tautomers of 4-Hydroxypyrimidine (A-Reduction, I^r-Representation)

	4PO		4HPcis	
	NH	ND	OH	OD
A/MHz	5974.858(6)[a]	5714.899(2)	6172.88(1)	6123.024(4)
B/MHz	2807.153(3)	2797.317(3)	2787.29(1)	2692.58(4)
C/MHz	1910.067(4)	1878.309(5)	1920.26(1)	1869.30(4)
Δ_J/kHz [b]	−0.130(8)	[−0.130][c]	−0.13(3)	[−0.13]
Δ_{JK}/kHz	−0.34(3)	[−0.34]	−0.3(1)	[−0.3]
Δ_K/kHz	−0.8(1)	[−0.8]	−0.8(2)	[−0.8]
δ_J/kHz	−0.059(5)	[−0.059]	−0.06(1)	[−0.06]
Δ_c/uÅ^2	−0.030(8)	−0.04(1)	−0.004(2)	0.13(15)
N[d]	46	11	18	7
σ/MHz [e]	0.05	0.07	0.03	0.11

[a] Error in parentheses are expressed in units of the last digit. [b] The δ_K quartic centrifugal distortion parameters have been fixed to zero because not determined in the fit. [c] Parameters in square brackets were fixed to the value of the corresponding normal species. [d] Number of rotational transition in the fit. [e] rms deviation of the fit.

the lengths of the arrows, are based on literature data for 2PO/2HP and uracil and on the results of this work for 4HP/4PO/6PO.

We can see that the complexity of the tautomeric equilibrium increases proportionally to the number of hydroxyl groups and of imino nitrogens in the systems. Maybe accidentally, the number of tautomers is equal to the sum of the ring nitrogens plus the number of phenolic (or ketonic) oxygens. While a rich scientific literature is available for both 2PO/2HP and uracil, no data are reported for the intermediate system 4HP/4PO. For this reason, we focused our research on this system and analyzed its rotational spectrum, as described below.

Experimental Section

A sample of 4-hydroxypyrimidine was purchased from Aldrich and was used without further purification.

The free jet absorption millimeterwave (FJAMMW)[22] spectrum has been recorded in the 60−78 GHz frequency range. The sample, solid at room temperature, was heated to a temperature of 150 °C. He, at a pressure of 300 mbar, was used as the carrier which was passed over the heated sample. The mixture was then expanded through a nozzle with a diameter of 0.2 mm reaching an estimate rotational temperature of 10−20 K.

The monodeuterated species was generated by flowing He over D_2O (99% supplied by Promochem GmbH) at room temperature and then over the heated sample.

Dipole moment measurements have been carried out in a standing waveguide heated cell in the frequency range 26.5−39.6 GHz with a Stark spectrometer based on a Hewlett-Packard 8400C instrument.

Results and Discussion

(A) Theoretical Calculations. We carried out preliminary density functional calculations at the B3LYP/6-31G** level of approximation[23] to have values of the relative energies of the possible conformational/tautomeric species and their optimized geometries. The calculations also provided the rotational constants and components of the dipole moment along the principal axes. These data, useful for microwave spectroscopy studies, are shown in Table 1. In addition, the quadrupole coupling constants of the two ^{14}N nuclei have been calculated. Since we did not observe the corresponding quadrupole hyperfine structure, they are given as Supporting Information.

Two conformers of the hydroxyl tautomer, cis and trans according to the orientation of the hydroxyl group with respect to the adjacent nitrogen atom, and two keto forms have been found to be stable minima. The corresponding sketches are shown at the top of Table 1.

The B3LYP/6-31G** energy order is $E_{6PO} > E_{4HPtrans} > E_{4HPcis} > E_{4PO}$, with a large energy gap between the first and the second pair. For this reason, species 6PO ($E \approx 42$ kJ mol^{-1}) and 4HPtrans ($E \approx 28$ kJ mol^{-1}) are unlikely to be observed with our spectrometer. For the two most stable species, we also performed MP2/6-311++G** calculations. No large changes have been obtained for the rotational constants or dipole moment components with respect to the B3LYP/6-31G** values, but the energy order has been reversed. The experiments will judge which of the two theoretical approaches is the correct one.

(B) Assignment of the Spectra. The theoretical values of the rotational constants of Table 1 have been used to predict

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Table 3. Principal Axis System Coordinates (Å) of the Imino Hydrogen (H_N, Keto Forms) or Hydroxyl Hydrogen (H_O, Hydroxyl Forms)[a]

		H_N (keto forms)			H_O (enolic forms)	
		a	*b*		*a*	*b*
r_s (exptl)		±0.781(2)	±1.972(1)		±2.522(1)	±0.844(2)
B3LYP/6-31G**	4PO	0.775	1.983	4HPcis	2.534	0.849
	6PO	−1.784	−2.048	4HPtrans	2.603	−0.863

[a] The coordinate *c* is zero, for both forms, from the condition of planarity.

Table 4. Comparison between Experimental and Calculated Values of the Dipole Moment Components of 4PO and 4HPcis

	4PO			4HPcis		
	μ_a/D	μ_b/D	μ_{TOT}/D	μ_a/D	μ_b/D	μ_{TOT}/D
exptl[a]	1.70(2)	1.19(2)	2.08(3)	1.01(2)	0.69(3)	1.22(2)
B3LYP/6-31G**	−1.63	−1.51	2.22	1.24	0.52	1.34

[a] Absolute value of the dipole moment components. The value of the μ_c dipole moment component has been fixed to zero.

the spectra. We started the research for 4PO, which is expected to be the most stable species, and with a high value of the μ_b dipole moment component. We could assign several strong μ_b-R-type transitions, with *J* rotational quantum number ranging from 6 to 12 and very high K_{-1} (e.g., $6_{5,1}-5_{4,2}$). Then, we moved to the measurement of the transitions of the weaker μ_a-R-type bands at higher rotational quantum number with *J* in the range from 12 to 15. Subsequently, several unassigned weaker transitions were identified as μ_b-R-type transitions of 4HPcis. Their intensities were about one-fifth of those of the corresponding lines in the 4PO spectrum. We measured several μ_a-R-type transitions also for this species. Finally, to locate the hydroxyl (or the imino) hydrogen, we searched for and assigned the rotational spectra of the deuterated isotopologues for both tautomers.

Some of the μ_b-R-type lines show a broadening because of the quadrupole effects of the two ^{14}N nuclear spins ($I = 1$). However, the hyperfine structure was not reliably resolvable for any transition. All transitions, given as Supporting Information, have been fitted with Watson's Hamiltonian (A-reduction, I^r-representation),[24] and the obtained spectroscopic constants are reported in Table 2 for both tautomers. There we also give the values of the inertial defects, Δ_c. They are very close to zero, according to the planarity of both tautomers.

(C) Conformational and Structural Data. The two sets of experimental rotational constants fit satisfactorily the theoretical values for the 4PO or 6PO and 4HPcis or 4HPtrans species. Relying on the calculated values of energy, it is very plausible to assume that the observed spectra belong to the 4PO and 4HPcis forms, respectively. However, an experimental and independent confirmation of this choice comes from the substitution coordinates of the hydroxyl or imino hydrogens, derived from the rotational constants of the monodeuterated species by applying Kraitchman's equations.[25] The results in term of Cartesian coordinates are given in Table 3.

One can see that the experimental values of the r_s coordinate fit those calculated for species 4PO and 4HPcis. However, while the discrimination between the two keto forms is neat, the one between 4HPcis and 4HPtrans is based on a 0.1 Å difference in the *a*-coordinate. However, either the theoretical energy values or the chemical intuition that the O−H···N interaction favors energetically 4HPcis leaves little doubt on the conformational assignment.

(D) Dipole Moments. The values of the dipole moment components of 4PO and 4HPcis were determined from the analysis of the second-order Stark effect on six rotational transitions, three for each species. The measurements were performed adding a dc voltage to the square wave modulation field, and the cell was calibrated with the 3←2 transition of OCS ($\mu = 0.71512$ D[26]). The experimental slopes $\Delta\nu/E^2$ can provide the values of the dipole moment components through

$$\Delta\nu/E^2 \propto \mu_a^{\ 2}(\Delta A^a + \Delta B^a M^2) + \mu_b^{\ 2}(\Delta A^b + \Delta B^b M^2) \quad (1)$$

where ΔA^a, ΔA^b, ΔB^a, and ΔB^b, calculated with computer program MODTOP,[27] are the differences between the Stark coefficients[28] of the two levels involved in the rotational transition. They were calculated using the effective rotational constants reported in Table 2.

In the case of 4PO, we identified some transitions for which several Stark lobes overlap each other, forming the so-called "giant Stark lobes".[29] For these transitions, the ΔA^a and ΔA^b coefficients, the effects of which are independent from the quantum number *M*, are much larger than the ΔB^a and ΔB^b coefficients, which determine the dependence on M^2. The observed $\Delta\nu/E^2$ slopes, the Stark coefficients, and the results of the least-squares fits are given as Supporting Information.

The obtained dipole moment components are compared in Table 4 to the B3LYP/6-31G** theoretical values.

(E) Tautomeric Equilibrium. An estimate of the relative energy of the 4PO and 4HPcis tautomers can be obtained by combining relative intensity measurement with the values of the dipole moment components of Table 4, according to the following equation:[30]

$$\Delta E_{0,0} = kT \ln[(I_{4PO}\Delta\nu_{4PO}\mu_{g,4HP}^{\ \ 2}\gamma_{4HP}\nu_{4HP}^{\ \ 2})/(I_{4HP}\Delta\nu_{4HP}\mu_{g,4PO}^{\ \ 2}\gamma_{4PO}\nu_{4PO}^{\ \ 2})] \quad (2)$$

where $\Delta E_{0,0}$ [$=E_{0,0}(4HP) - E_{0,0}(4PO)$] is the energy difference between the two tautomers in their rotational and vibrational ground states. I, $\Delta\nu$, γ, μ_g, and ν are peak height, half-height width, line strength, dipole moment component (g = a or b), and transition frequency, respectively, of the considered transition.

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Table 5. Comparison between Experimental and Theoretical Values of the Relative Energy of 4HPcis and 4PO[a]

	$\Delta E_{0,0}$/kJ mol^{-1}
exptl	2.0(9)
B3LYP/6-31G**	4.2
MP2/6-311++G**	−5.2

[a] $\Delta E_{0,0} = E_{0,0}(4HP) - E_{0,0}(4PO)$.

From the analysis of several pairs, close in frequency, of μ_a and μ_b-type rotational transitions, a value of $\Delta E_{0,0} = 2.0(9)$ kJ mol^{-1} has been obtained, showing that 4PO is the global minimum. The pairs of transitions used for intensity measurements are given as Supporting Information.

As shown in Table 5, the experimental result agrees better with the B3LYP/6-31G** calculations, while the MP2/6-311++G** prediction is off by more than 7 kJ mol^{-1}. It is interesting to note that the use of the theoretical values of the dipole moment components (with errors up to 0.3 D) would have generated a high imprecision in the $\Delta E_{0,0}$ tautomeric energy difference.

Conclusions

The tautomeric equilibrium in 2PO/2HP, 4HP/4PO/6PO, and uracil can be schematically represented as $-\mathrm{N{=}C(OH)}- \leftrightarrow -\mathrm{NH{-}C({=}O)}-$. Such equilibrium is shifted to the left for 2PO/2HP but neatly to the right for uracil, as shown by numerous scientific reports mentioned above. No data were available on the system 4HP/4PO/6PO, whose complexity is intermediate with respect to 2PO/2HP and uracil. In this paper, we report the first spectroscopic investigation of 4HP/4PO/6PO. Its rotational spectrum shows that the tautomeric equilibrium is already shifted to the right (in favor of the keto form); more precisely, the keto form 4PO is the most stable species. It appears that the stability of the keto with respect to the enolic forms increases with the number of ring nitrogen atom and with the number of OH available to permute in keto groups.

These experimental results show that MP2/6-311++G** theoretical calculations fail in determining the energetic features of this kind of tautomeric equilibria. The fact that MP2 calculations overestimate the stability of the enol form was already outlined in the case of 2PO/2HP.[21]

No lines were found corresponding to the 6PO and 4HPtrans species, in agreement with the very high energy, as predicted by the theoretical claculations.

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Supporting Information Available: Complete ref 23; Table 1s: Calculated B3LYP/6-31G(d,p) quadrupole coupling of the four stable species of 4-hydroxypyrimidine and its keto forms. Nitrogen N1 is that at the bottom in the sketches; Table 2s: Frequencies (ν, MHz) and discrepancies between experimental and calculated values ($\Delta\nu$, MHz) of the measured transitions of 4PO and 4HPcis; Table 3s: Stark coefficients of 4PO and 4HPcis; Table 4s: List of the pairs of transitions which have been used for the relative intensity measurements. The peak's areas and frequencies are given. This material is available free of charge via the Internet at http://pubs.acs.org.

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