Proportions of Species Observed in Jet Spectroscopy−Vibrational Energy Effects: Histamine Tautomers and Conformers

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Abstract: It has been found possible to understand the observed relative abundances of the several different tautomers and conformers observed in the millimeter-wave jet spectroscopy of histamine by using ab initio energy calculations at the MP2/6-311++G(d,p) level and including an estimate of thermal free energies. Conformational relaxation, previously found to be important for several molecules of comparable complexity, has been found to have a significant role in determining the relative abundances of histamine species detectable in the cooled jet, the present example being indicative that this phenomenon is expected to influence generally the interpretation of multiconformational jet spectra. The need to include free-energy corrections is also noteworthy. The thermal equilibration of histamine tautomers in the gas phase within the millimeter-wave spectrometer prior to jet expansion has been confirmed experimentally by comparing abundances measured with high surface area and low surface area inlet systems. Thus, it is valid to use relative abundances to indicate relative free energies. The presented ab initio calculations yield spectroscopic constants (e.g., planar moments) that tighten the identification of the four species detected in jet spectroscopy. In particular, the identity of the least abundant species (3 G-Ic) is confirmed. Nitrogen quadrupole coupling constants computed at the MP2/6-311++G(d,p) level are in noticeably better agreement with experiment than previously reported calculations, it being noteworthy that they require no scaling for the ^{14}N nuclear quadrupole moment.

Introduction

When molecules of moderate complexity are studied spectroscopically using a supersonic expanding jet technology to obtain better resolved and structurally simpler high-resolution spectra, it is often necessary to consider which of a range of species, conformers and tautomers, might be involved. Many studies have been reported in which ab initio molecular orbital calculations of varying degree of complexity have been used to help clarify the issue. In a number of cases, a group of species has been selected as being the most stable according to the calculations, and the spectroscopic results have been interpreted with the help of these calculations.

However, from time to time some disagreement has arisen because species that are predicted to fall in the group of lowest energy have not always been detected in the expanding supersonic jet. This has led to debate as to whether the ab initio results are sufficiently reliable or whether other factors enter into consideration.

Recently,[1] attention has been drawn to the fact that in the supersonic expansion some species may relax to other, more stable species, this being feasible if the barrier to the relaxation is low enough. This has led to the resolution of several "discrepancies" between theory and experiment.

There is a further complication in that the Gibbs free energies of species should be used when predicting relative proportions present at thermodynamic equilibrium. However, when considering relaxations of species to more stable forms during the jet expansion, it is the relative positions of zero-point energy (ZPE) levels (i.e., the Gibbs energy with *T* approaching absolute zero) that should be computed. Most of the theoretical studies have been based just on electronic energies, it being assumed that zero-point effects and thermal free energy corrections will be very similar for different conformers.

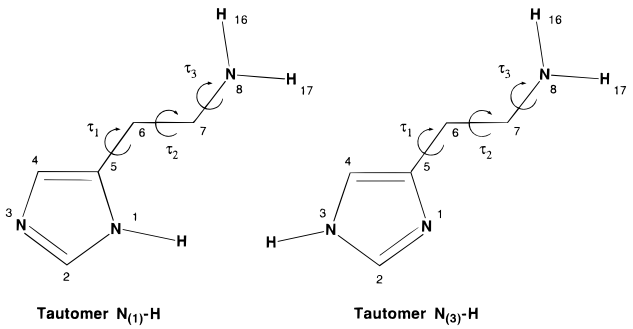

Figure 1. Atom numbering scheme and labeling of torsional angles in the two tautomers of histamine.

Among various molecules of biological interest, histamine[2] (Figure 1) was studied by jet spectroscopy before consideration of conformer relaxation and of free energy corrections had been

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(1) Godfrey, P. D.; Brown, R. D.; Rodgers, F. M. *J. Mol. Struct.* **1996**, *376*, 65−81.

(2) Histamine (Figure 1) has been the subject of a voluminous literature because of its biological importance. Its biological activity has stimulated various studies of its tautomerism and geometry, including NMR studies in solution,[3] an X-ray crystallographic study,[4] a structural study of jet-cooled histamine vapour by millimetre-wave spectroscopy[5] and by molecular orbital calculations of varying sophistication.[5,6] The results of these studies were not completely in agreement as to which tautomer/conformer is the most stable. However the millimeter-wave study of the vapour pointed to species $N_{(1)}$ G IVa and $N_{(3)}$ G Ib (see Figure 2)[7] as being the most stable, with two other species also being present in moderate amounts. These findings were roughly in qualitative harmony with the most complete ab initio molecular orbital calculations reported to that time[3] in that the four species identified experimentally were among the five lowest energy species as predicted by both RHF/3-21G and RHF/6-31G ab initio calculations.

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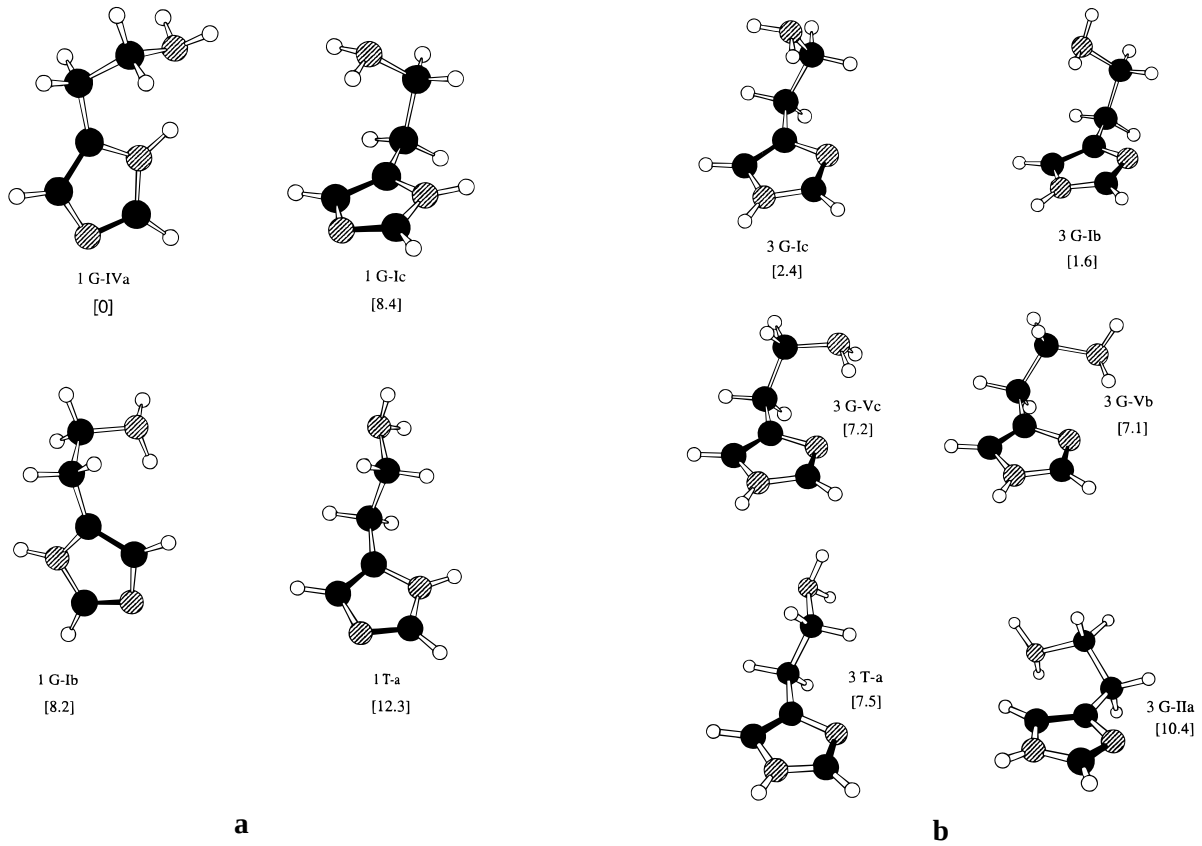

Figure 2. Conformers of the two tautomers of histamine. Figures in brackets are relative free energies in kJ mol^{-1} (see Table 1).

shown to be significant. The study of histamine species left two questions unanswered:

(i) Why do we observe four species of histamine in the jet spectrometer when ab initio calculations indicate that just one species is appreciably more stable than the rest?

(ii) Why do we not detect one of the predicted most stable conformers (GVb of tautomer $N_{(3)}$, see Figure 2b), while we see others of similar predicted stability?

In the interim, there have been advances in computer technology that make it feasible to use more elaborate theoretical approximations when investigating conformer stabilities and to explore whether the effects of molecular vibrations significantly influence the theoretical interpretations. The present study also contributes some indications of the magnitude of error bars for computing molecular energies of molecules of the complexity of histamine.

Reliability of MO Calculations

It has become feasible to carry through full optimization ab initio molecular orbital calculations on moderately sized molecules using larger basis sets and including allowance for electron correlation. This less severe level of truncation should reduce the uncertainties that might come from the error bars associated with the MO calculations.

There is still only a rough estimate of how reliable ab initio calculations are at various degrees of complexity for the prediction of relative stabilities. For small molecules, one can make a direct comparison between calculations and experimental estimates of energies;[8] however, experimental relative energies for conformers of molecules the size of histamine are not available at the level of accuracy needed to establish useful error bars for predicting stabilities of conformers. We therefore have to resort to indirect inferences such as observed relative abundances of species in jet spectroscopy, where several assumptions (e.g., thermodynamic equilibrium, that anharmonicities of vibrations can be neglected when estimating free energies, etc.) are needed when estimating relative stabilities. For example, it has become accepted that calculations at the MP2/6-31G(d,p) level are necessary in order to obtain results for the relative electronic energies of different conformers of molecules of the complexity of glycine, phenylethylamine, etc. that are reliable enough to pinpoint the specific species observed in jet spectroscopy. However, the question of the size of the error bars for such calculations relating to the conformers and tautomers of molecules of similar complexity has not been answered adequately. Our working hypothesis is that in

(3) Ganellin, C. R.; Pepper, E. S.; Port, G. N. J.; Richards, W. G. *J. Med. Chem.* **1973**, *16*, 610−616; Ham, N. S.; Casy, A. F.; Ison, R. R. *J. Med. Chem.* **1973**, *16*, 470−475; Byrn, S. R.; Graber, C. W.; Midland, S. L. *J. Org. Chem.* **1976**, *41*, 2283−2288; Reynolds, W. F.; Tzeng, C. W. *Can. J. Biochem.* **1977**, *55*, 576−578; Wasylishen, R. E.; Tomlinson, G. *Can. J. Biochem.* **1977**, *55*, 579−582.

(4) Bonnet, J. J.; Ibers, J. A. *J. Am. Chem. Soc.* **1973**, *95*, 4829−4833.

(5) Vogelsanger, B.; Godfrey, P. D.; Brown, R. D. *J. Am. Chem. Soc.* **1991**, *113*, 7864−7869.

(6) Kang, S.; Chou, D. *Chem. Phys. Lett.* **1975**, *34*, 537−541; Topiol, S.; Weinstein, H.; Osman, R. *J. Med. Chem.* **1984**, *27*, 1531−1534; Gresh, N.; Claverie, P.; Pullman, A. *Theor. Chim. Acta* **1984**, *66*, 1−20; Smeyers, Y. G.; Romero-Sanchez, F. J.; Hernandez-Laguna, A. *J. Mol. Struct.* **1985**, *123*, 431−442; **1990**, *207*, 157−167; Topiol, S. *J. Comput. Chem.* **1987**, *8*, 142−148.

(7) Although the standard nomenclature for histamine is either 1H-imidazole-4-ethanamine or 1H-imidazole-5-ethanamine depending upon the tautomer considered, all previous papers dealing with theoretical calculations on histamine and also the biochemical literature adopt the numbering system used here, where the skeleton numbering does not change with tautomerism. We use the notation $N_{(1)}$−H and $N_{(3)}$−H or, where unambiguous, $N_{(1)}$ or $N_{(3)}$ to designate the tautomers, or sometimes just 1 or 3 when different conformers are being discussed.

(8) Hehre, W. J.; Radom, L.; Schleyer, P. v. R.; Pople, J. A. *Ab Initio Molecular Orbital Theory*; Wiley: New York, 1986; p 294.

favorable cases the error bars can be inferred by requiring experimental observations to be in agreement with the calculated energies.

Earlier data for small polyatomic molecules[8] based on the MP2/6-31G(d) level of theoretical sophistication (but with geometry optimization at a lower level) show errors in predicted relative energies of structural isomers of up to some tens of kJ mol^{-1} but do not give any indication of the error bars for predicting relative energies of different conformers of the same structural isomer. Prediction of *absolute* energies of small polyatomics to ± 2 kJ mol^{-1} has been shown[9] to require refinements substantially beyond the MP2/6-31G(d,p) level. However, there does not appear to be any definitive study of the errors associated with the presumably less demanding task of predicting the *relative* energies of molecular conformers and particularly for molecules of the moderate complexity exemplified by histamine.

A previous study[1] of six molecular conformer pairs for which experimental and theoretical data on relative energies were available provided some evidence that relative energies of conformers were predictable, using full geometry optimizations at the MP2/6-31G(d,p) level, to within about ± 2 kJ mol^{-1}. In the interconversion of conformers, of course, no normal chemical bonds are broken or formed, whereas for relative energies of tautomers or other structural isomers, where the bonding topology changes, we anticipate a lower level of reliability. It seems desirable to get further performance data for the ab initio calculations based on somewhat larger molecules, such as histamine.

Conformer Relaxation. The answer to question ii might lie in some analogous work on the moderately sized molecules glycine, alanine, and glycolic acid.[1,10] These studies initially indicated that ab initio calculations, even with relatively modest basis sets such as RHF/4-31G, reliably predict which particular conformer is the most stable of all (but see below). Nevertheless, where several conformers had been identified by spectroscopy, there were some discrepancies as to which conformers, other than the most stable, should be detected. These discrepancies have been shown to be attributable to the relaxation of some conformers to other more stable conformers in supersonic expanding jets. The proviso is that the potential barrier to relaxation is low enough (no more than $\sim$1000 cm^{-1}).[1]

For the earlier calculations on histamine, the computed electronic energy differences (more than 10 kJ mol^{-1} for the 6-31G basis calculations, more than 14 kJ mol^{-1} for the 3-21G basis) implied that only the single most stable species would be present in detectable amounts. The former calculations predicted energy differences about $^3/_4$ the size of the latter, suggesting that more elaborate calculations might produce results more consistent with the observation of several species. More recent calculations by Nagy et al.[11] on "the most stable forms" of tautomer 1 and tautomer 3 at the MP2/6-31G*//RHF/6-31G* and MP2/6-311++G**//RHF/6-31G* levels have indeed shown a diminution in the predicted energy difference; with the inclusion of corrections for the free energy at 298 K, the difference between the two most stable species was reduced to 7.5 kJ mol^{-1}, still, however, too great to explain the spectroscopic observations. Moreover, their most stable forms were selected on the basis of earlier calculations of Vogelsanger et al.;[5] however, the current study shows that when larger basis sets and electron correlation corrections are used, the most stable form of tautomer 3 is predicted to be 3 G-Ib, not 3 G-Vc (the studies by Nagy et al. of relative stabilities in solution should be revised, using the species of tautomer 3 now known to be the most stable).

Herein, we further explore the problem that three species of the less stable tautomer of histamine have been identified by jet spectroscopy but they do not correspond to those predicted to have the lowest electronic energies at the RHF/6-31G level of ab initio calculation. Our aim is to investigate this anomaly and simultaneously to obtain further indications of the reliability (in terms of error bars to energy calculations) of the ab initio calculations at the MP2/6-31G(d,p) and MP2/6-311++G(d,p) levels. We focus on the two main questions enunciated above.

In addressing question i, we hypothesise that the appearance of comparable amounts of the two tautomers in the jet spectroscopy is to be attributed to residual errors in calculations of tautomeric relative free energies. For completeness, we considered the alternative hypothesis that the histamine vapor above the melt had not reached tautomeric equilibrium prior to the jet expansion. Experiments are reported below, designed to test this hypothesis.

To address question ii, regarding the three conformers of tautomer 3 observed in the jet spectrum, we have undertaken MP2/6-31G(d,p) and MP2/6-311++(d,p) level calculations to establish whether calculations at these higher levels might provide an explanation of the observations.

Methodology

In the present study, our strategy is to seek an explanation of observed relative abundances of conformers via theoretical arguments that involve computing total electronic energies and to consider further ab initio-based theoretical estimates of ZPE corrections and Gibbs free energy corrections. ZPE corrections may be decisive because directions of conformer relaxations in the supersonic jet are dependent on relative positions of ZPE levels (see below); Gibbs energy corrections may be influential in predicting the equilibrium proportions of species in the preexpansion chamber of the jet system. These corrections require predictions of vibrational frequencies for which other error bars apply, including the approximations of (a) using harmonic frequencies even though, for some of the vibrations, the anharmonicities are considerable or even extreme (in the case of many large-amplitude motions, which make the largest vibrational contributions to Gibbs energies) and (b) the use of an empirical scaling factor. In exploring agreement between theory and experiment in this manner, we indirectly derive indications of error bars associated with the calculations.

Ten histamine species, selected as the six lowest energy species in previous calculations, and including three others from the next most stable group of four were subjected to complete geometry optimizations. Total electronic energies were obtained at the MP2/6-31G(d,p) and MP2/6-311++(d,p) levels using the Gaussian 94 package.[12] ZPE and Gibbs energy corrections (harmonic approximation) were similarly computed at the MP2/6-31G(d,p) level. It has become customary to scale computed frequencies to bring them more into line with experiment, the widely adopted scaling factor for computations at the RHF/6-31G(d,p) level being 0.8912. However, there seems to be no widely agreed upon factor for frequencies computed at the MP2/6-31G(d,p) level. We therefore utilized a study[13] of the fundamental

(9) Pople, J. A.; Head-Gordon, M.; Fox, D. J.; Raghavachari, K.; Curtiss, L. A. *J. Chem. Phys.* **1989**, *90*, 5622.

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Table 1. Relative Stabilities of Histamine Tautomers and Conformers[a]

species	E(MP2)/ E_h	rel ΔE(MP2)/ kJ mol^{-1}	rel ΔE (MP2 + ZPE)	rel ΔG/ kJ mol^{-1}	mol fract. in jet after expansion from 130 °C[b] (a)[c,d]	(b)
1 G-IVa	−359.265904	.000	.000	.000	0.372	0.37
1 G-Ic	−359.260340	14.609	12.711	8.400	0.030	
1 G-Ib	−359.260551	14.056	12.140	8.233	0.032	
1 T−a	−359.258212	20.197	17.566	12.323	0.009	
3 G-Vc	−359.261747	10.916	9.716	7.241	0.13[c]	0.15
3 G-Vb	−359.261707	11.020	9.681	7.135	0.000[d]	
3 G-Ic	−359.261769	10.858	8.763	2.441	0.09[c]	0.11
3 G-Ib	−359.262339	9.316	7.179	1.576	0.277[d]	0.37
3 T−a	−359.260295	14.728	12.150	7.450	0.040	
3 G-IIa	−359.260243	14.863	13.072	10.384	0.017	

[a] Electronic energies calculated at MP2/6-311++G(d,p) level, ZPE and Gibbs corrections at MP2/6-31G(d,p) level. [b] Predicted from relative free energies with appropriate allowances[c,d] for relaxation in the jet expansion. (b) Experimental, ref 3. [c] The sum of these two mole fractions is predicted to be 0.222. Bearing in mind the very close similarity of the respective conformer predicted Gibbs energies, we have apportioned this sum between 3 G-Vc and 3 G-Ic to most nearly match observed relative abundances (see discussion of relaxation in text). [d] Note that allowance for the relaxation in the jet of 3 G-Vb to 3 G-Ib has been made.

Table 2. Barrier Heights for Conformer Relaxations for Histamine-3

relaxation	barrier height kJ mol^{-1}	barrier height cm^{-1}
3 G-Vb → 3 G-Ib	3.7	310
3 G-Vb → 3 G-Vc	10.4	870
3 G-Vc → 3 G- Ic	5.7	480
3 G-Vc → 3 G- Ib	21.3	1780

frequencies of pyrrole and imidazole, in which frequency calculations at the MP2/6-31G(d,p) level were reported, to derive an average scaling factor of 0.991 and adopted this value in present calculations. The results are collected in Table 1.

For the five species found in the present calculations to be of lowest free energies (see Figure 2), we studied the barriers for interconversion of the conformers. These were estimated in each case by selecting the torsional angle that seemed to best represent the main coordinate change for the particular interconversion. Constrained optimizations at a series of values for that particular coordinate (τ_1 for G-Vb → G-Ib and G-Vc → G-Ic; τ_3 for G-Vb → G-Vc; both τ_1 and τ_3 for G-Vc → G-Ib, where τ_1, τ_3 are defined in Figure 1) were then run at the MP2/6-31G(d,p) level. Resultant total electronic energies as a function of this torsional parameter were utilized to obtain an estimate of the barrier heights. Improved estimates of the barriers were obtained by single-point constrained optimized energy calculations at the MP2/6-311++G(d,p) level for τ values corresponding closely, as judged from the more detailed MP2/6-31G(d,p) calculations, to values at the top of the barriers. Results are listed in Table 2. Detailed searching to find the precise transition states was not feasible computationally, and thus, values in Table 2 must be regarded as merely reasonable estimates of reaction pathways and barriers. This should be adequate for the present purposes because we need only to classify barriers as low (less than about 1000 cm^{-1}) or high (above about 1000 cm^{-1}).

Experiments on the jet spectroscopy of histamine were conducted using a spectrometer of the type described by Brown et al.[14] Argon was passed over molten histamine at 130 °C in the preexpansion chamber. In a separate experiment, the inlet system between the preexpansion chamber and the jet was packed with glass fragments to expedite the establishment of tautomeric equilibrium in the hot vapor. The flow rate of the carrier gas through the vaporization chamber corresponded to a residence time of about 15 s; the glass packing represented less than 10% of the chamber volume which would not have appreciably reduced the residence time of histamine; indeed, the residence time was probably increased by adsorption/desorption processes of histamine on the packing, akin to gas-chromatographic processes. Because the spectrometer sensitivity is frequency-dependent, we selected four transitions of 1 G-IVa and four of 3 G-Ib (see Table 3), measured ratios of signal strengths, and converted these to molar ratios by using the peak absorption coefficients that are provided by program WANGSR (the program that we have used over many years in the analysis of rotational spectra of asymmetric rotor molecules) together with the experimental values of dipole components previously published.[5]

Quadrupole coupling calculations used the current version of our QUAD4 code.[15] This uses standard first-order quadrupole coupling theory for up to four quadrupolar nuclei.

Results and Discussion

Tautomers. Our experimental observations on using a packed vaporization chamber to expedite the establishment of tautomeric equilibrium by heterogeneous catalysis showed no significant change in relative signal strengths, as shown in Table 3. If the only experimental uncertainties came from the estimates (given in parentheses in Table 3) of uncertainties in measured signal strengths, i.e., around 2−16% for the unpacked chamber measurements, 7−25% for the packed chamber, then the values of S/α would mirror these uncertainties, being constant to that degree for each species. The entries in columns 6 and 7 show greater variation. This is to be attributed to some instrumental variation in sensitivity with frequency, and thus, the averages are seen to show rather larger rms values (14−17%). The ratio of packed to unpacked chamber is identical for the two species, well within the experimental uncertainty.

We conclude that equilibrium is already established in the unpacked chamber because the alternative, that equilibrium is approached very slowly even in a packed vessel, seems much less plausible, given that the chamber residence time is so long compared with expected rates of tautomerization.

Relative Energies. The predicted relative energies of different species of histamine (and presumably those of other species of similar complexity) depend noticeably on the theoretical method used. This is illustrated in Figure 3a for the five most stable forms of histamine. While all methods predict the G-IVa species of tautomer 1 to be the most stable, relative energies predicted for the four most stable conformers of tautomer 3 change substantially as we increase the size of basis set. It is surprising that the inclusion of diffuse functions appreciably changes the electronic energy of 3 G-Vc compared with that of the other conformers of tautomer 3 (see Figure 3a).

(13) Nagy, P. I.; Durant, G. J.; Hoss, W. P.; Smith, D. A. *J. Am. Chem. Soc.* **1993**, *115*, 2912−2922.

(14) Brown, R. D.; Crofts, J. G.; Godfrey, P. D.; McNaughton, D.; Pierlot, A. P. *J. Mol. Struct.* **1988**, *190*, 185−193.

(15) Blackman, G. L.; Bolton, K.; Brown, R. D.; Burden, F. R.; Mishra, A. *J. Mol. Spectrosc.* **1973**, *47*, 457−468. Modernized and improved by P. D. Godfrey.

Table 3. Relative Proportions of Histamine Tautomers[a]

transition	frequency/ GHz	S/μV a	S/μV b	$100 \times \alpha/$ cm^{-1} c	S/α a	S/α b
histamine 1 G-IVa						
19(2,17) ← 18(2,16)	48.885	1.53(3)	1.09(7)	5.81	26.3	18.8
21(1,21) ← 20(1,20)	48.905	2.00(5)	1.48(10)	5.49	36.4	27.0
21(0,21) ← 20(0,20)	48.908	1.94(8)	1.55(17)	5.49	35.3	28.2
19(3,16) ← 18(3,15)	50.337	1.63(5)	1.28(10)	5.68	28.7	22.5
					av: 31.7	24.1
histamine 3 G-Ib						
19(1,19) ← 18(1,18)	48.756	0.61(4)	0.48(4)	1.31	46.6	36.6
19(0,19) ← 18(0,18)	48.854	0.60(7)	0.47(6)	1.31	45.8	35.9
19(1,18) ← 18(1,18)	49.749	0.41(4)	0.36(4)	1.30	31.5	27.7
19(2,17) ← 18(2,16)	49.963	0.44(7)	0.32(8)	1.25	35.2	25.6
					av: 39.8	31.5

[a] S, signal strength. α, absorption coefficient: a, with unpacked pre-expansion chamber; b, with glass-packed pre-expansion chamber; c, theoretical absorption coefficient at a temperature of 10 K based on MP2/6-31G(d,p) value of μ_a: 1 G-IVa, 5.78 D; 3 G-Ib, 2.62 D. Ratio of S/α for [1 G-IVa]/[3 G-Ib]: unpacked, 0.80; packed, 0.77.

When we include corrections for ZPE or for free energy at the temperature used in the spectroscopic study (see Figure 3b), the relative stabilities are again appreciably changed. As outlined below, this materially changes our interpretation of the observations of species in the jet spectroscopy of histamine.

At the simplest level of theory reported (RHF/3-21G), species 3 G-IIa (not shown in Figure 3) becomes the fourth most stable but is predicted to be much less stable, relatively, at the MP2/6-31G(d,p) level. The two most stable conformers of tautomer 3 (on the basis of free energies estimated at 403 K), 3 G-Ic and 3 G-Ib, are less stable relative to other conformers when assessed just on total electronic energies, even at the MP2/6-31G(d,p) level. As can be seen from Figure 3b, the change in predicted stabilities of 3 G-Vc and 3 G-Vb is about 8−9 kJ mol^{-1} relative to 1 G-IVa and is mostly a thermal free-energy effect, ZPEs contributing only about 2 kJ mol^{-1}. The lowest computed frequencies for 3 G-Ic and 3 G-Ib are 22 and 29 cm^{-1}, respectively, while the lowest for 3 G-Vc and 3 G-Vb are 48 and 44 cm^{-1}, respectively. Since the lowest frequencies make the greatest contributions to free energies, it is tempting to attribute the effect of the free-energy corrections primarily to these low frequencies, which appear to be torsional motions involving the exocyclic amino group. In the first two conformers, an amino hydrogen appears to be weakly interacting with the imidazole ring via π-electrons, whereas in the last two conformers the interaction, presumably a stronger hydrogen-bond type interaction and so "stiffening" the torsional motion, is with the ring nitrogen lone pair.

Given that the experimental observations on histamine indicate equal equilibrium abundances of 1 G-IVa and 3 G-Ib, we conclude that the predicted relative stabilities are in error by about 1.5 kJ mol^{-1} according to free-energy calculations based on MP2/6-311++G(d,p) electronic energy calculations and about 7 kJ mol^{-1} according to MP2/6-31G(d,p) calculations. However, when we use the relative free energies to compute mole fractions of the different species in the jet preexpansion chamber, the predicted and observed mole fractions for 1 G-IVa are in remarkable agreement (see Table 1). Of course this single observation is of little statistical significance and points to the need for further test cases to consolidate our error bar estimates.

This kind of error bar clearly needs further study, but it seems likely that some of the uncertainty arises because for the calculation of vibrational frequencies and free-energy corrections the harmonic approximation was used and a single scaling factor of 0.991 was applied to all of the predicted frequencies. One might have suspected that bends and torsions would require

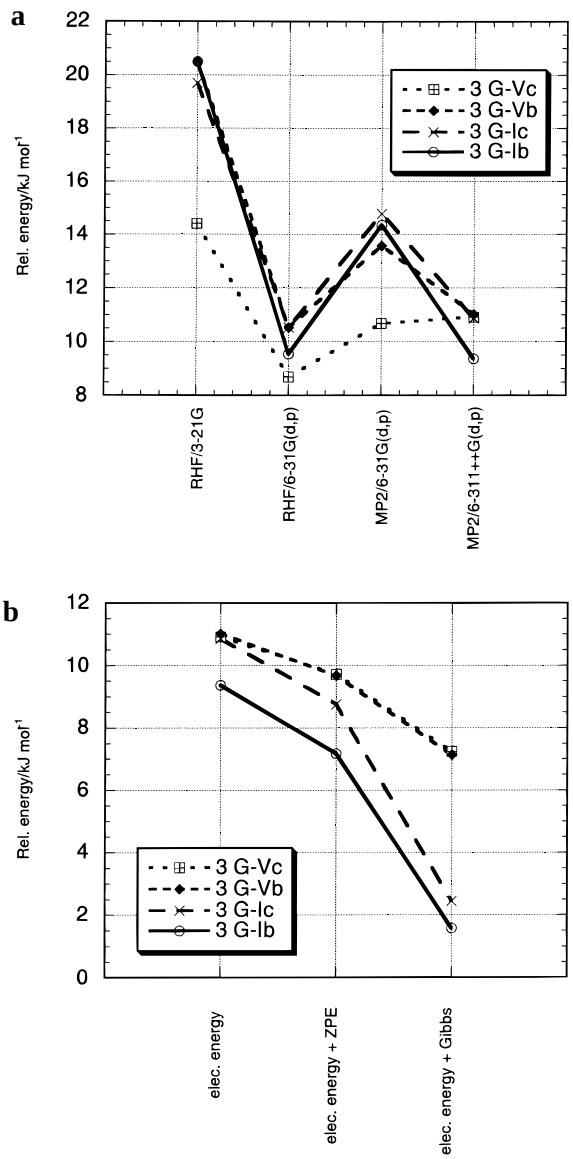

Figure 3. (a) Total electronic energies, relative to 1 G-IVa, by different MO methods for the four most stable conformers of histamine $N_{(3)}$−H. (b) Effects (computed at the MP2/6-31G(d,p) level) of vibrational energy corrections (zero-point energies) and Gibbs energy corrections on relative stabilities of the four conformers (MP2/6-311++G(d,p) electronic energies).

different scaling factors from stretches, and while the scaling factor needs further consideration, we suspect that a substantial contribution to the energy error is the effect of large-amplitude vibrations of considerable anharmonicity. Such LAMs are common in molecules of complexity similar to that of histamine but are not so common in the very small polyatomic species that have been widely used in testing the reliability of ab initio calculations.

The vibrational contributions in thermal free-energy corrections have been computed from frequencies derived by the ab initio calculations that used the harmonic approximation.[16] We might question whether for severely anharmonic vibrations this might introduce additional errors above that of the base estimate of total electronic energies. For molecules as large as histamine, there is no theoretical procedure that we are aware of to provide vibrational energy data at approximation levels better than the harmonic approximation. However, some feel for the magnitude of anharmonicity influences on free-energy computations can be gained by calculations for smaller molecules that have a very anharmonic vibration. We evaluated the contribution to ΔG from the ν_{12} mode of formamide for which there is experimental data and a semirigid bender calculation on this vibration. At 403 K, the contribution to ΔG is -1.32 kJ mol^{-1} computed from the experimental frequencies and -1.42 kJ mol^{-1} derived from the harmonic approximation (using the $1 \leftarrow 0$ transition frequency rather than the curvature for the bottom of the potential function, the latter being dependent upon how one fits a function to this vibrational mode). Some of the LAMs present in the various histamine species will differ markedly from this example in formamide, but the latter indicates that errors in using the harmonic approximation are probably in the range of tenths of a kJ mol^{-1} so that comparative free-energy calculations probably have errors well below 1 kJ mol^{-1}.

Conformers. Second, we must consider why, of the different tautomer 3 species observed, 3 G-Ib was a little more abundant than 3 G-Vc and 3 G-Ic and that 3 G-Vb was not detected. The predicted free energies at 403 K span a range of only 2.5 kJ mol^{-1}, and we might accept that the residual uncertainties in the calculations mean that discrepancies between observed and predicted abundances are within the error range of the calculations. We note, however, that the two conformers observed to be in greatest abundance, 3 G-Ib and 3 G-Ic, are predicted to have the lowest free energies, hinting that the calculations are reliable enough to select the most stable conformers even when such small energy differences are involved. However, then we have to consider why of the two conformers predicted to have virtually identical free energies, 3G-Vc and 3 G-Vb, the former is observed experimentally in similar abundance to 3 G-Ic while the latter is not detected in the expanding jet. Since in our studies of other biomolecules we have concluded that conformer relaxation can occur if the barrier to relaxation is low enough, we therefore explored possible relaxations of 3 G-Vb to either of the lower energy conformers.

For relaxation in the jet expansion, we need to consider the relative ZPEs of the different conformers. These are shown in Figure 3b which indicates that we need to consider 3 G-Vb → 3 G-Ib, 3 G-Vc → 3 G-Ic and 3 G-Vb → 3 G-Ic. The estimated barrier heights are given in Table 2.

3 G-Vb → 3 G-Ib. The barrier is low, less than 350 cm^{-1}. This is not unexpected because the relaxation requires only the rotation of the side chain against the ring (i.e., change in τ_1). The barrier is low enough that we expect conformer relaxation in the spectrometer jet; indeed, it is low enough that 3 G-Vb and 3 G-Ib should possibly be considered to be a single species with a double-minimum torsional potential, the energies of the lower vibrational states being affected by tunneling. In the jet expansion, we expect relaxation to the lowest vibrational state which would resemble 3 G-Ib rather than 3 G-Vb. The spectroscopic constants should therefore be nearer to those of 3 G-Ib, in harmony with the spectroscopic observations on histamine vapor.

3 G-Vc → 3 G-Ic. This is a situation analogous to the previous one, and therefore we expect the relaxation barrier to be relatively low. The ab initio calculations confirm this (Table 2). Thus, since 3 G-Ib is predicted to be of ZPE lower than 3 G-Vc, we might expect that the latter conformer will relax to the former in the jet expansion.

Why then do we observe conformer 3 G-Vc? We have to make the plausible assumption that the difference between the calculated zero-point energy levels for these two species is in error by ca. 1 kJ mol^{-1} and that they are actually very close in energy. In such circumstances, both species would be seen in the jet spectrum even though they have only a small barrier to interconversion.

3 G-Vb → 3 G-Vc. The relaxation of G-Vb to G-Vc involves the rotation of the amino group with an eclipse of the side-chain C−C by an N−H and therefore would be expected to have a higher barrier than for the previous cases. We estimate the barrier to be at least 870 cm^{-1}, which is consistent with a lack of relaxation of this kind. The estimated value is a little below the suggested critical value of 1000 cm^{-1}, but since error bars for this energy criterion are around ± 200 cm^{-1}, we consider the result to be in harmony with the spectroscopic observations of no relaxation.

Finally, we note that, had we sought understanding of the spectroscopy using just the total electronic energies rather than free energies, we would have been faced with the prediction, even at the MP2/6-31G(d,p) level, that 3 G-Vc was the most stable conformer of tautomer 3 (by 3−4 kJ mol^{-1}) and would have had to lamely accept that the error bars in the ab initio calculations were responsible for the disagreement between theory and experiment. Even at the MP2/6-311++G(d,p) level, the use of just total electronic energies alone would have required us to assume error bars of at least 10 kJ mol^{-1} to bring theory and experiment into harmony for both tautomers. We suggest that histamine is unlikely to represent an isolated instance and that one cannot comfortably rely on the use of just electronic energies in theoretical interpretations of jet spectroscopy observations on conformers and tautomers.

Identity of Species C/T 4. In the original spectroscopic identification of the four species in the vapor phase, the species C/T 4 was tentatively identified as the gauche Ic conformer of tautomer 3 (i.e., $N_{(3)}$−H G-Ic). The evidence was that the rotational constants, inertial defect, and dipole moment components matched those computed at the RHF/6-31G level for this species better than for any others. The quadrupole hyperfine structure of some spectral lines was reasonably similar to that computed for the G-Ic conformer, but the extent of agreement between the two was not great enough to be considered as definite evidence of identification. The more elaborate calculations on which the present study are based give us an opportunity of reconsidering this identification of the fourth species.

Table 4 lists the rotational constants computed for all four species of histamine together with their dipole moment components for comparison with the experimental values. Figure

(16) See, for example, Herzberg, G. *Infrared and Raman Spectra of Polyatomic Molecules*; D. Van Nostrand: 1945, Chapter 5.

Table 4. Comparison between Experiment[a] and Theory at the RHF/6-31G(d,p), MP2/6-31G(d,p), and MP2/6-311++G(d,p) Levels for the Assigned Histamine Species

quantity	experiment	RHF/ 6-31G(d,p)	(o − c)	MP2/ 6-31G(d,p)	(o − c)	MP2/ 6-311++G(d,p)	(o − c)
Histamine N(1)−H GIVa							
A, MHz	4952	5063	−111	4962	−10	4936	16
B, MHz	1392	1390	2	1410	−18	1401	−9
C, MHz	1141	1144	−3	1151	−10	1147	−6
μ_a, D	5.4	5.5	−0.1	5.8	−0.4	5.8	−0.4
$(\mu_b^2 + \mu_c^2)^{1/2}$, D	0.9	1.2	−0.3	1.1	−0.2	1.0	−0.1
μ_{total}, D	5.5	5.6	−0.1	5.9	−0.4	5.8	−0.3
Histamine N(3)−H G-Ib							
A, MHz	4506	4662	−156	4487	19	4461	45
B, MHz	1332	1311	21	1350	−18	1349	−17
C, MHz	1273	1251	22	1292	−19	1290	−17
μ_a, D	2.3	2.6	−0.3	2.6	−0.3	2.5	−0.2
$(\mu_b^2 + \mu_c^2)^{1/2}$, D	1.8	2	−0.2	2	−0.2	2.0	−0.2
μ_{total}, D	3	3.3	−0.3	3.3	−0.3	3.2	−0.2
Histamine N(3)−H GVc							
A, MHz	4763	4912	−149	4779	−16	4781	−18
B, MHz	1374	1366	8	1390	−16	1379	−5
C, MHz	1156	1149	7	1161	−5	1154	2
μ_a, D	3.9	4.2	−0.3	4.4	−0.5	4.2	−0.3
$(\mu_b^2 + \mu_c 2)^{1/2}$, D	1.8	1.9	−0.1	2	−0.2	2.2	−0.4
μ_{total}, D	4.3	4.7	−0.4	4.8	−0.5	4.8	−0.5
Histamine N(3)−H GIc							
A, MHz	4482	4617	−135	4476	6	4431	51
B, MHz	1313	1301	12	1341	−28	1329	−16
C, MHz	1273	1262	11	1298	−25	1292	−19
μ_a, D	3.1	3.6	−0.5	3.9	−0.8	3.5	−0.4
$(\mu_b^2 + \mu_c^2)^{1/2}$, D	2.9	3.1	−0.2	3	−0.1	3.2	−0.3
μ_{total}, D	4.2	4.8	−0.6	4.9	−0.7	4.7	−0.5

[a] *A*, *B*, and *C* are rotational constants, and μ is the dipole moment; all are derived from the analysis of rotational spectra.

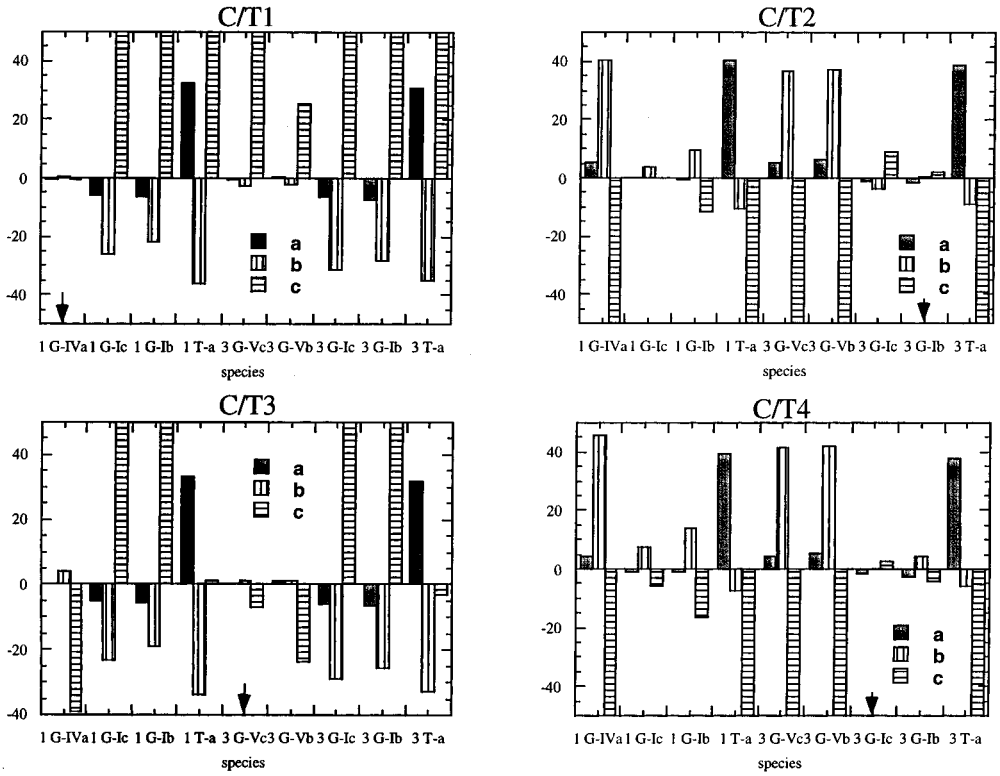

Figure 4. Comparison of planar moments derived from microwave spectroscopy and from ab initio calculations [MP2/6-311++G(d,p)] for the four species (C/T 1, C/T 2, C/T 3, and C/T 4) detected by Vogelsanger et al.[5] Arrows indicate previous identifications by Vogelsanger et al. (consolidated by current work). Plotted values are % deviations of theory from experiment; note that some deviations are off-scale (values up to 300%) but truncation was used so that the smallest deviations are more visible.

4 shows the % difference between computed [MP2/6-31G(d,p)] and experimental values of planar moments for all four detected species and the most stable conformers and tautomers. Consideration of planar moments ($P_a = (-I_A + I_B + I_C)/2$ and cyclic permutations for P_b and P_c) is perhaps most intuitively useful when considering molecular shapes because such moments are indicative of the distances of atoms from each of the inertial planes (*bc*, *ca*, and *ab*).

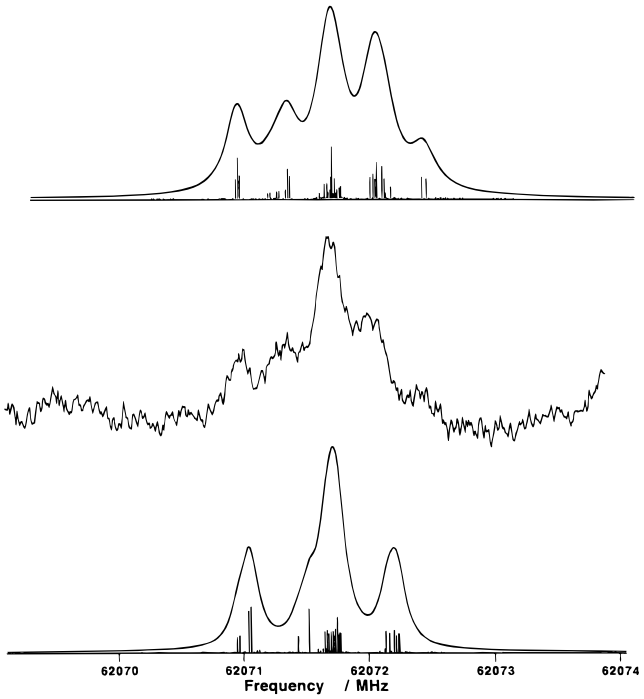

Figure 5. Comparison of computed (top and bottom) and observed (middle) shapes of the $22_{2,21} \leftarrow 21_{1,20}$ transition for species 3 G-Ic. The lowest multiplet was computed at the RHF/6-31G level, the uppermost at the MP2/6-311++G(d,P) level.

If we consider first the three species for which identification is well established, it is seen that the rotational constants computed at the MP2/6-31G(d,p) level coincide with the rms deviation of 1.2%. When the older theoretical data at the RHF/6-31G level are used, the rms error is 1.8% and we have also now found that at the RHF/6-31G(d,p) level the rms error is 1.9%. However, the prediction of *A*, (a measure of the smallest dimension of the species) shows an improvement by an order of magnitude when electron correlation is included at the MP2 level. The planar moment deviations in the graphs within Figure 4, labeled C/T1, C/T2, and C/T3, clearly show that the three species are best identified with $N_{(1)}$ G-IVa, $N_{(3)}$ G-Ib, and $N_{(3)}$ G-Vc.

For the fourth species, the agreement with rotational constant values calculated for the 3 G-Ic species at the MP2/6-311++G-(d,p) level is within 1.5%, making the identification more convincing than before. (This is also clear from the planar moment deviations shown in the graph within Figure 4, labeled C/T4.)

The computed dipole moment components show improved agreement with experimental values for all four species when we use the MP2/6-311++G(d,p) level.

So far as the quadrupole hyperfine structure of the $22_{2,21} \leftarrow 21_{1,20}$ transition is concerned, the shape computed for the multiplet agrees better with the experimental shape for the 3 G-Ic conformer than for any of the other low-energy conformers. The agreement between computed and observed shape of the multiplet, shown in Figure 5, is significantly better than was reported in our earlier study. It should be noted that in the present quadrupole multiplet computations, a best-estimate value for the nitrogen quadrupole moment (2.3×10^{-26} cm^2)[17] was employed, whereas our earlier calculations employing smaller basis sets used a smaller scaled value recommended by Brown and Head-Gordon[18] for calculations using more limited bases. Our study of the multiplets for the histamine species now represents a good example of the utility of using quadrupole multiplets as an important independent means of identifying species from a list of plausible alternatives.

We infer that the identification for all four species now seems definite and the identification of $N_{(3)}$ G-Ic should no longer be considered tentative.

Conclusions

Ab initio calculations at the MP2/6-311++G(d,p) level coupled with appropriate thermal free-energy corrections are successful in identifying four species of histamine from the twenty possible stable species as those observed in jet spectroscopy. No other histamine species would have been detected in the spectroscopic studies because the least abundant of the four species (3 G-Ic) had lines of low signal-to-noise ratio when the spectrometer was operating in searching mode. The species predicted to be next in abundance, 3 T-a, less abundant by a factor of about 3, would not have been detected in the spectroscopic search for species. As part of the predictive process, the relaxation of two species partly or wholly to other species is predicted. Predictions based on total electronic energies alone or calculations that use smaller basis sets or ignore electron correlation are less satisfactory. The level of reliability of computed relative free energies is about ± 1.5 kJ mol^{-1} for tautomers and about ± 1 kJ mol^{-1} for conformers, based on this single example.

The proportions of the two tautomers of histamine, histamine 1 and histamine 3, observed in rotational jet spectroscopy represent the position of tautomeric equilibrium in the vaporized sample before jet expansion. Ab initio calculations at the MP2/6-311++G(d,p) level predict that in such an equilibrated sample, 38% of the histamine detected in the jet spectrum would be 1 G-IVa in startling agreement with the experimental finding of 37% that Vogelsanger et al. reported. Calculations at the less elaborate MP2/6-31G(d,p) level predict a greater proportion of tautomer 1, indicating a probable error in such energy calculations of about 6 kJ mol^{-1}.

For tautomer $N_{(1)}$, the detection of only conformer 1 G-IVa implies that it is the most stable conformer of this tautomer. Ab initio calculations of conformer energies of $N_{(1)}$ at the MP2/6-311++G(d,p) level are in full accord with observations, predicting that 1 G-IVa is at least 8 kJ mol^{-1} lower in free energy (at least 10 kJ mol^{-1} at the MP2/6-31G(d,p) level) than any other conformer of histamine $N_{(1)}$.

For tautomer $N_{(3)}$, the detection of conformers 3 G-Ib, 3 G-Vc, and 3 G-Ic in the proportions 10:4:3 can be reconciled with ab initio calculations that predict relative free energies of the conformers in the vapor before jet expansion and also predict relaxation of 3 G-Vb to 3 G-Ib in the jet expansion. It is noteworthy that agreement between theory and experiment is attained only if Gibbs free-energy corrections are included. Calculated total electronic energies alone do not satisfactorily explain the observations (and if calculations at the MP2/6-31G-(d,p) level are used we would wrongly predict 3 G-Vc to be the most stable by 3-4 kJ mol^{-1}). The fact that conformers 3 G-Ic and 3 G-Ib are more stable than 3 G-Vc is attributable to the greater negative Gibbs free-energy contribution from the very low-frequency vibrations of the first two as compared with

(17) Scuseria, G. E.; Schaefer, H. F. *J. Chem. Phys.* **1987**, *87*, 4020−4024.

(18) Brown, R. D.; Head-Gordon, M. P. *Mol. Phys.* **1987**, *61*, 1183−1191.

(19) Gai, F.; Hasson, K. C.; McDonald, J. C.; Anfinrud, P. A. *Science* **1998**, *279*, 1886−1891.

that of 3 G-Vc despite the first two having somewhat higher electronic energies.

At first sight, the observation of 3 G-Vc is not explained by our calculations, these implying that it should relax to 3 G-Ic in the jet. However if the calculations have a residual error and these two conformers are actually of almost equal energy, then the observation of both is understandable. The implication is an error of ca. 1 kJ mol^{-1} in the computed relative zero-point energy levels.

The success of the MP2/6-311++G(d,p) level ab initio calculations in predicting observed mole fractions of vapor phase species is excellent, almost within the error bars for extimating relative abundances of species, for histamine. The calculated relative energies for both conformers and tautomers are reliable to about ± 1.5 kJ mol^{-1} at this level, but studies of additional compounds will be needed to see whether this is a typical figure.

The ab initio calculations at the MP2/6-31G(d,p) level, taken together with analogous studies of several similar flexible molecules, predict relative electronic energies and barriers to interconversion of conformers with a reliability of about ± 200 cm^{-1}. Calculations of differences in free energies of tautomers are uncertain to at least ± 6 kJ mol^{-1}.

The present study, which demonstrates that one must sometimes go beyond just the consideration of potential energy surfaces and at least focus on ZPEs or sometimes Gibbs free energies, applies more widely than just jet spectroscopy of moderate-sized molecules. In a randomly selected example, we note that a recent review of the photoisomerization of retinal in bacteriorhodopsin discussed the dynamics of excited states purely in terms of the potential energy curve although shallow minima, and hence large-amplitude motion, would seem to be involved.

The present study also consolidates the identification of the species C/T 4 as the gauche conformer Ic of tautomer 3, i.e., $N_{(3)}$ G-Ic. This has been achieved by comparison of planar moments derived from microwave spectroscopy with theoretical values predicted at the MP2/6-311++G(d,p) level.

The excellent agreement between computed and experimental quadrupole hyperfine multiplets for the various histamine species indicates that calculations, at the MP2/6-311++G(d,p) level, of electric field gradients for N atoms, combined with the best current estimate of the quadrupole moment of the nitrogen nucleus to estimate coupling constants, represent a valuable tool for identifying conformers and tautomers of nitrogen-containing species.

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