Spectroscopic Studies on Vapour Phase Tautomerism of Natural Bases Found in Nucleic Acids

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Natural Purines and Pyrimidines, Infrared Spectra, Ultraviolet Spectra, Gas Phase Tautomerism, Heats of Vaporization

Infrared absorption spectra, in the vapour phase, have been recorded in the regions of NH, OH and carbonyl stretching frequencies, for a series of 1-substituted uracils and 9-substituted adenine and hypoxanthine, formal analogues of the natural nucleosides of uracil, adenine and hypoxanthine found in DNA and/or RNA. A number of related analogues was also examined, including the N-methyl derivatives of the known mutagen and chemotherapeutic agent, 5-fluorouracil.

The infrared absorption spectra provide unequivocal evidence for the existence of 1-substituted uracils as the 2,4-diketo tautomer, of 9-substituted hypoxanthine as the 6-keto tautomer, and of 9-substituted adenine as the 6-amino tautomer. These are the known predominant tautomeric forms in solution, so that the gas phase results are in sharp contrast with those for other nitrogen heterocycles, where the tautomeric equilibrium constants may differ by several orders of magnitude in going from solution to the vapour phase. The significance of these results is evaluated in relation to the types of heterocyclic bases found in natural nucleic acids, and to concepts of spontaneous and induced mutations in terms of mis-pairing.

The ultraviolet absorption spectra of the various compounds have also been examined in the gas phase, but proved of relatively limited use in studies on tautomeric equilibria under these conditions.

Heats of vaporization have been determined for most of the compounds examined. In particular the heat of vaporization for 1,3-dimethyluracil, which is incapable of association by hydrogen bonding in the condensed phase, is much lower than for uracil and 1(3)-methyluracils which can associate by hydrogen bonding.

Introduction

The well-known effects of molecular environment on tautomeric equilibria in nitrogen heterocycles, illustrated in some instances by solvent-induced changes [1–3], are particularly striking when examined in the gas phase [4] relative to that in solution. For example, in the case of 2-oxopyridine, the keto form appears to be predominant in aqueous medium, whereas in the vapour phase the keto and enol forms are present in approximately equal proportions [5–7].

In the pyrimidine series, 2-oxopyrimidine and 4-oxopyrimidine [7], and analogous derivatives [8], are also predominantly in the keto form in aqueous medium, whereas in the gas phase the former is exclusively in the enol form, while the latter exhibits an approximately 1:1 ratio of the keto and enol forms. These findings are particularly relevant to the results of quantum chemical calculations, which should theoretically be applicable only to molecules in a vacuum (*i. e.* in the gas phase), but are generally compared, and are usually in agreement, with experimental results in aqueous medium.

The experimentally observed very marked departure of K_T in the gas phase, as compared to aqueous medium, for 2- and 4- oxopyrimidines, and a number of other heterocyclic system [7], raises the question as to the possible behaviour of some natural pyrimidines and purines, particularly those which are normal constituents of RNA and/or DNA, i. e. 1-substituted pyrimidines and 9-substituted purines. Attempts to examine this problem are necessarily limited by the ability to bring a given compound into the gas phase, requiring elevated temperatures that may lead to thermal decomposition.

In a search for such model compounds, we have found, in agreement with previous observations [9–11], that uracil and methylated uracils can be examined in the gas phase under conditions where they are fully stable. The same applies to some methylated hypoxanthines, including 9-ethylhypoxanthine,

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an analogue of inosine, which is found in the anticodons of some tRNA's, as well as some methylated adenines, including 9-methyladenine. We report here on the gas phase tautomerism of a number of the foregoing, as well as one example of a mutagenic base analogue, 5-fluorouracil, which is capable of being incorporated into RNA.

Experimental

Uracil was a product of Waldhof-Pharmacia (Stuttgart, GFR); 5-fluorouracil was from Hoffman-LaRoche; and the following products from Cyclo Chemical Corp. (Los Angeles, Calif., USA): 9-ethylhypoxanthine, 7-methylhypoxanthine, 9-methyladenine, 3-methylaldenine, 1-methyladenine, 1,3,6-trimethyluracil.

The N-methylated derivatives of uracil, 5-fluorouracil and 6-chlorouracil were prepared as elsewhere described [12 – 14]. Standard methylation procedures [15] were employed for the preparation of 1,7-dimethylhypoxanthine, 1,9-demethylhypoxanthine, 1,9-dimethyladenine, N^6, 9-dimethyladenine, and 6-methoxy-9-methylpurine.

All compounds were chromatographically homogeneous in several solvent systems and were also checked by ultraviolet spectroscopy in aqueous medium.

Infrared spectra were recorded on a Zeiss (Jena, GDR) UR-20, and ultraviolet absorption spectra on a Zeiss Specord UV-Vis.

Vapour phase infrared spectra in the region of NH and OH stretching frequencies, and UV spectra, were obtained with the aid of cylindrical quartz cells 2.5 cm or 1.8 cm in diameter, and 7 cm or 3 cm path-length. The sample material was introduced through a side tube which was sealed off after evacuation of the system to a pressure $\leqq 10^{-5}$ mm Hg. The cell (and an identical control) was mounted in a metal asbestos-lined chamber heated by a spiral, and controlled through a thermoregulator. A copper-constantan thermocouple attached to the cell wall was used to monitor the temperature continuously during an experiment, with a variation not exceeding ± 5 °C. The quartz windows of these cells were sufficiently transparent down to 2550 cm^{-1}.

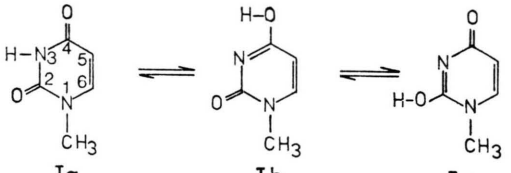
Measurements below 2000 cm^{-1} were carried out with a demountable cell consisting of a stainless steel cylinder 2 cm in diameter, and 9 cm path-length, fitted with NaCl windows. Temperature control with this cell was less precise, the observed variation during an experiment being 10 – 15 °C.

With the exception of uracil, the spectra were examined under conditions of equilibrium between melted sample and the vapour; and also when the sample was fully transferred to the vapour phase, so as to make possible measurements of integral absorption coefficients of NH bands. Identical spectra were obtained under both conditions. In all instances, following completion of an experiment, the cell was cooled and the sample contents checked for possible thermal decomposition by UV spectroscopy and, occasionally, by chromatography. Results reported below, with two exceptions, pertain only to those cases where such thermal decomposition, or intramolecular rearrangement, was absent.

Results and Discussion

Uracil and some derivatives

N_1-substituted uracils may theoretically adopt one, or a mixture, of three tautomeric forms (**I a**, **I b**, **I c**), whereas uracil itself could also conceicably exist in the dihydroxy form **I d**, as shown in Scheme 1.



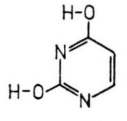


Fig. 1 exhibits the vapour phase spectra, in the region 3400 – 3600 cm^{-1} embracing NH and OH stretching frequencies, of uracil, 1-methyluracil, 3-methyluracil, and the 1-methyl and 3-methyl derivatives of 5-fluorouracil and 6-chlorouracil. The most striking feature of all these spectra, in comparison with those for pyridone-2 and 2- and 4-oxopyrimidine analogues [7, 8], is the total absence

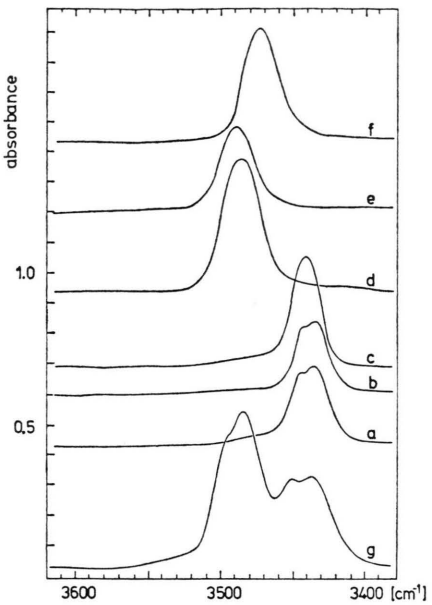


Fig. 1. Infrared absorption spectra, in the vapour phase, in the region of NH and OH stretching frequencies, of uracil and some substituted uracils. Figures in brackets are temperatures in the cell employed to bring the substance into the gas phase; (a) 1-methyluracil (230 °C); (b) 1-methyl-5-fluorouracil (225 °C); (c) 1-methyl-6-chlorouracil (210 °C); (d) 3-methyluracil (245 °C); (e) 3-methyl-5-fluorouracil (230 °C); (f) 3-methyl-6-chlorouracil (225 °C); (g) uracil (275 °C).

Note: In this, and subsequent, figures, the heights of the absorption bands for different substances are not strictly comparable, since the amount of material in the vapour phase varies. For some measurements of integral molar absorption coefficients, see text.

of any absorption attributable to an OH stretch, pointing to the existence of all of these in the diketo form. In further agreement with this is the fact that all exhibit characteristic NH stretching vibrations.

The 1-methyl and 3-methyl derivatives of uracil, 5-fluorouracil and 6-chlorouracil exhibit NH bands at about 3440 cm^{-1} and 3480 cm^{-1}, respectively; whereas the parent uracil displays, as might be anticipated, both bands (Fig.1, Table I). The spectra of the 1-methyl and 3-methyl derivatives permit of the assignment of the 3486 cm^{-1} band in uracil to the N_1H stretch, and the 3445 cm^{-1} band to the N_3H stretch. In the case of 1,3-dimethyluracil (not shown), no absorption is detectable in this region. It should also be noted that a 5-fluoro or 6-chloro substituent does not affect the frequency of the N_3H band, and only slightly modifies the N_1H band frequency.

Table I. Infrared absorption bands (in the region of NH and OH stretching vibrations), and principal ultraviolet long-wavelength absorption bands, of uracil and N-methylated uracils and 5(6)-halogenouracils in the vapour phase, as well as heats of vaporization for the various compounds.

Compound	Infrared spectra		Ultraviolet	ΔH_{vap} [kcal/mol]
	$\nu(N_1-H)$ [cm^{-1}]	$\nu(N_3-H)$	λ_{max} [nm]	
Uracil	3486	3445	256	32±2[a]
1-Methyluracil	—	3439	256	25±2
1-Methyl-5-fluorouracil	—	3438	263	30±2
1-Methyl-6-chlorouracil	—	3440	260	26±2
3-Methyluracil	3485	—	245	18±2
3-Methyl-5-fluorouracil	3489	—	256	19±4
3-Methyl-6-chlorouracil	3472	—	248	25±2
1,3-Dimethyluracil	—	—	256	11±1[b]

[a] Clark *et al.* (ref. [9]) report a value of 20 kcal/mol.
[b] Clark *et al.* (ref. [9]) report a value of 22 kcal/mol.

For 1(3)-methyluracils, which are most easily brought into the vapour phase, total vaporization of a known quantity of each of them made it possible to evaluate the molar integral absorption coefficients of the N_3H and N_1H stretching bands, 5.3×10^6 cm mol^{-1} and 7.5×10^6 cm mol^{-1}, respectively. It is of some interest that for the parent uracil, where a direct comparison is possible, the ratio of the corresponding coefficients for these bands is about 1.3.

The IR spectrum of 1-methyluracil was additionally examined in the region of carbonyl stretching frequencies, and exhibited an intense band with a maximum at about 1740 cm^{-1} and a $\nu_{1/2}$ of about 45 cm^{-1}. The large band width points to overlapping of two carbonyl frequencies, $C_2=O$ and $C_4=O$. In the case of a pyrimidine with a single carbonyl group, viz. 4-oxopyrimidine, the observed half-width of the carbonyl stretching band in the gas phase was 25 cm^{-1} [8]. Furthermore, in CCl_4 solution, where 1-methyluracil is known to exist in the diketo form, two carbonyl bands are observed, at 1695 cm^{-1} and 1720 cm^{-1}, with half-widths of 17 cm^{-1} and 16 cm^{-1}, respectively.

Heats of vaporization of uracil derivatives

For any of the foregoing compounds, the absorbance of the NH band(s) increases with an increase in temperature, which transfers more of the substance into the vapour phase. This temperature-induced increase in absorbance may be employed to evaluate the heat of vaporization of a given

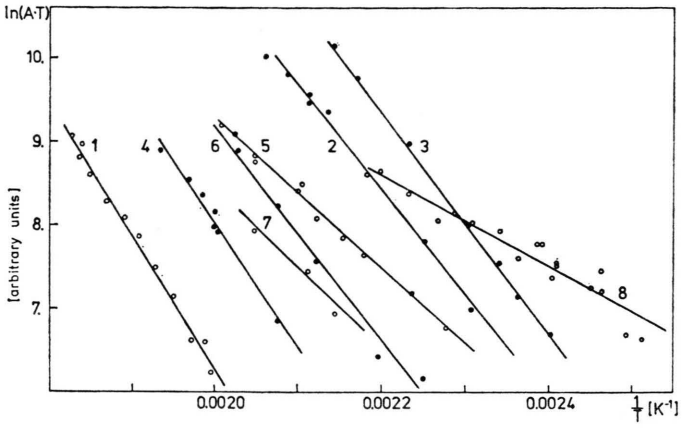


Fig. 2. Plots of ln $(A \cdot T)$ *vs* $1/T$ (where A is the absorbance of an NH stretch in the gas phase at an absolute temperature T) for: (1) uracil; (2) 1-methyluracil; (3) 1-methyl-6-chlorouracil; (4) 1-methyl-5-fluorouracil; (5) 3-methyluracil (6) 3-methyl-6-chlorouracil; (7) 3-methyl-5-fluorouracil; (8) 1,3-dimethyluracil (this compound, of course, has no NH stretch and a CH stretch at 2961 cm^{-1} was used to follow the dependence of A on T).

compound from the slope of the plot of ln p *vs* $1/T$, where p is an experimental parameter proportional to the vapour pressure, and T the absolute temperature. The product of the integral intensity of the NH band, A (or the height of the band maximum when the band-width is not significantly temperature-dependent, as was the case here) and temperature T, *i. e.* $A \cdot T$, is, in fact, proportional to p. Such plots of ln $(A \cdot T)$ *vs* $1/T$ are shown for the individual derivatives in Fig. 2, and the resulting calculated values for the heats of vaporization in Table I. For 1,3-dimethyluracil, which exhibits no NH band, the measurements were based on the absorbance of the 2961 cm^{-1} band in the CH stretching region. Since this compound cannot associate by hydrogen bonding in the condensed phase, it would be expected to exhibit a lower value of ΔH_{vap} than the other derivatives. This measured value is, in fact, only 11 kcal/mol, as compared to a range of 18 – 31 kcal/mol for the other derivatives.

The difference in ΔH_{vap} between 1,3-dimethyluracil and uracil (or a monomethylated uracil) may be considered as the energy required for rupture of intermolecular hydrogen bonds in the condensed phase. It will be noted that this difference is about 20 kcal/mol for uracil and appreciably lower for most of the monomethylated uracils.

The heats of vaporization of uracil and 1,3-dimethyluracil had been previously evaluated by Clark *et al.* [9], using ultraviolet absorption spectroscopy. They obtained 20 kcal/mol for uracil and a somewhat higher value, 22 kcal/mol, for 1,3-dimethyluracil. We are at a loss to account for this discrepancy. It is our feeling that the inordinately high value for 1,3-dimethyluracil renders that for uracil equally doubtful.

UV absorption spectra

Fig. 3 exhibits the vapour phase UV spectra of some of the uracil derivatives. The similarity of the spectra for uracil, 1-methyluracil, 3-methyluracil, 1,3-dimethyluracil and 1,3,6-trimethyluracil (this compound was used here only for reference purposes) is clearly consistent with all of these being in the diketo from. It should, be noted that the dienol analogue **I d**, *i. e.* 2,4-diethoxypyrimidine, differs from all the former only in that it displays a clearly

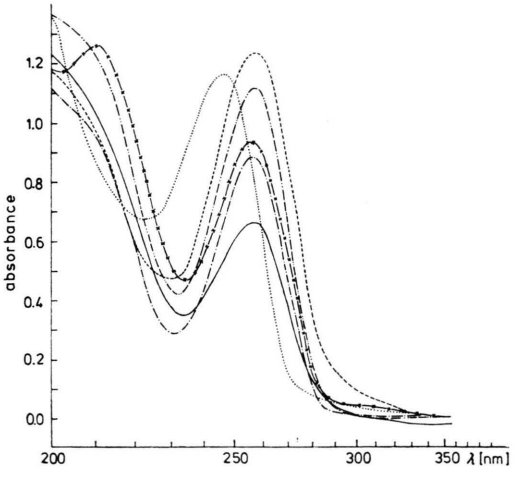


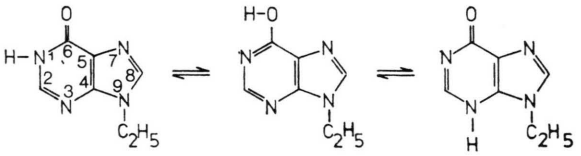
Fig. 3. Ultraviolet absorption spectra in the gas phase, at temperatures indicated in brackets, for: ———, uracil (260 °C); — — —, 1-methyluracil (270 °C); · · · ·, 3-methyluracil (270 °C); —·—·—, 1,3-dimethyluracil (210 °C); ——··——··, 1,3,6-trimethyluracil (234 °C); —×—×—, 2,4-diethoxypyrimidine (190 °C).

defined short-wavelength maximum at about 217 nm. However, the profiles of the short-wavelength absorption for the other derivatives clearly indicates that they must all exhibit similar maxima just below 200 nm. Consequently the UV spectra in this case, for uracil and its methylated derivatives, while consistent with the diketo forms for uracil and its monomethylated derivatives, do not by themselves provide convincing evidence for this. The same applies to some extent for the other systems described below, and emphasizes the essential utility of the IR data in such studies.

An additional point of some interest is that 1-methlyuracil, the long-wavelength maximum of which is markedly shifted to the red relative to the parent uracil in solution [12], is located in the same position as uracil in the gas phase. We have noted similar examples in the case of 5- and 6-halogeno substituted uracils. It follows that the gas-phase UV spectra may be of utility in assessing solvent effects on the spectra of these compounds.

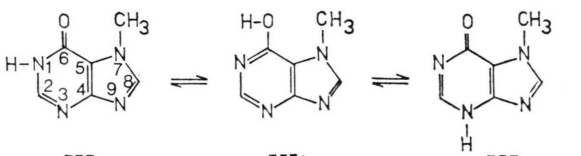
9(7)-Alkylhypoxanthines

9-Ethylhypoxantine, a formal analogue of inosine, possesses one mobile proton which may theoretically be located on N(1), N(3) or O^6, so that the possible tautomeric forms are as shown in Scheme 2.



IIa IIb IIc

An analogous situation prevails for 7-methylhypoxanthine which, although bearing no relation to any constituent found in natural nucleic acids, was considered a useful model for comparison purposes, following our observation that it could readily be brought into the gas phase. The possible tautomeric forms of this compound are shown in Scheme 3.



IIIa IIIb IIIc

The gas phase IR spectra of 9-ethylhypoxanthine and 7-methylhypoxanthine are depicted in Fig. 4. Each exhibits a single well-defined band in the NH stretching region at 3442 cm^{-1} and 3436 cm^{-1}, respectively, and no absorption in the OH stretching region. Both compounds also display an intense, symmetrical, band in the 1700 cm^{-1} region for a carbonyl stretching frequency (see also Table II).

Table II. Vapour phase infrared NH and carbonyl stretching frequencies, ultraviolet absorption bands, and heats of vaporization of N-methylated hypoxanthines.

Compound	Infrared bands [cm^{-1}]		Ultraviolet λ_{max} [nm]	ΔH_{vap} [kcal/mol]
	ν(NH)	ν(C=O)		
9-Ethylhypoxanthine	3436	1755	200, 243, 280	26±3[a]
6-Methoxy-9-methylpurine	—	—	200, 243	—
1,9-Dimethylhypoxanthine	—	1745	200, 243, 280	18±3
7-Methylhypoxanthine	3442	1722	200, 253	24±3
1,7-Dimethylhypoxanthine	—	1702	200, 253	—

[a] Clark *et al.* (ref. [9]) report a value of 20 kcal/mol.

The corresponding fixed 6-keto forms, 1,9-dimethylhypoxanthine and 1,7-dimethylhypoxanthine, show the expected absence of absorption in the NH stretching region, but display similar carbonyl bands, shifted slightly to lower frequencies as a result of N_1-methylation, but with integral absorptions similar to those of the parent 9(7)-alkyl derivatives. This is unequivocal evidence for the existence of both 7-methylhypoxanthine and 9-ethylhypoxanthine (and by inference inosine) in the 6-keto form in the gas phase, as previously reported for inosine in aqueous medium [16].

Corresponding UV spectra for the hypoxanthines in the vapour phase are shown in Fig. 5, from which it will be seen that the vapour spectrum of 9-ethylhypoxanthine is similar to that for the fixed keto form 1,9-dimethylhypoxanthine, and differs from that for the fixed enolic form 6-methoxy-9-methylpurine (Table II). In aqueous medium the differences between the first two and the latter are much less pronounced, as noted elsewhere [16]. Quite striking is the band at about 280 nm in the vapour phase spectra of 9-ethylhypoxanthine and 1,9-dimethylhypoxanthine, which almost disappears

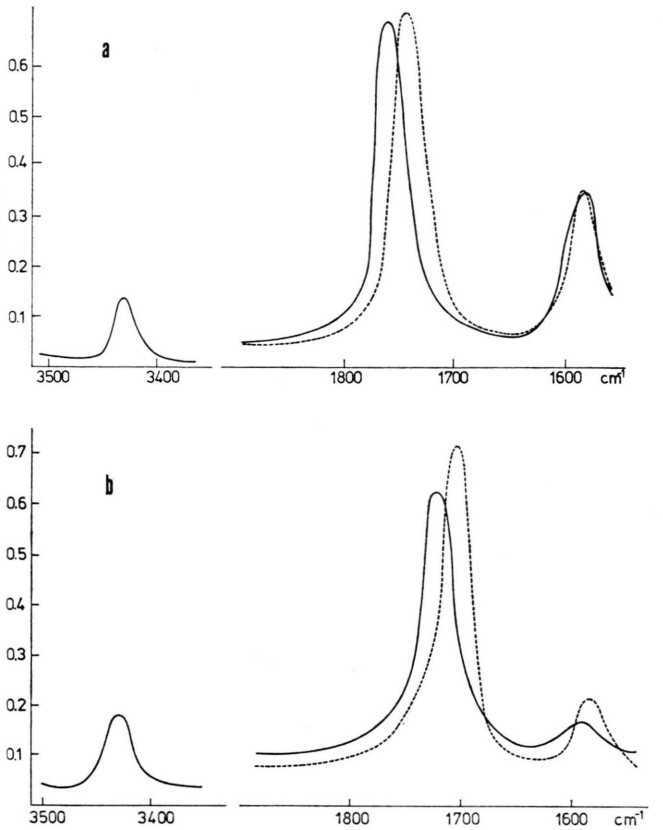


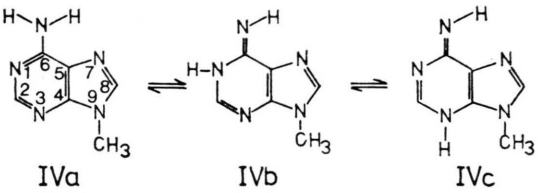
Fig. 4. Vapour phase infrared absorption spectra, in the regions of NH and C=0 stretching frequencies, at temperatures indicated in brackets, of: (a) ———, 9-ethylhypoxanthine (280 °C); — — —, 1,9-dimethylhypoxanthine (280 °C); (b) ———, 7-methylhypoxanthine (310 °C); — — —, 1,7-dimethylhypoxanthine (243 °C).

in the spectra of these compounds in solution [16]. The nature of this effect is clearly deserving of further study.

The UV spectra of 7-methylhypoxanthine and 1,7-dimethylhypoxanthine are also similar to each other, both in the vapour phase and in aqueous medium, consistent with each of them being in the 6-keto form. However the evidence for this conclusion is much more convincing from the IR data.

9-Methyladenine

This compound is a formal analogue of adenosine and may exist as the amino form **IV a**, and the imino forms **IV b** and/or **IV c** (Scheme 4). Its vapour phase spectrum in the NH stretching region (Fig. 6) exhibits two bands, at 3548 cm^{-1} and 3438 cm^{-1}, with relative integral intensities of 0.56.



In $CDCl_3$ solution the IR spectrum of 9-methyladenine exhibits the same two bands with analogous relative integral intensities. Bearing in mind that the amino tautomer **IV a** has been unequivocally established as the predominant form for 9-methyladenine (and adenosine) in both aqueous and non-aqueous media [17, 18, 20], the foregoing bands in the gas phase spectrum clearly correspond

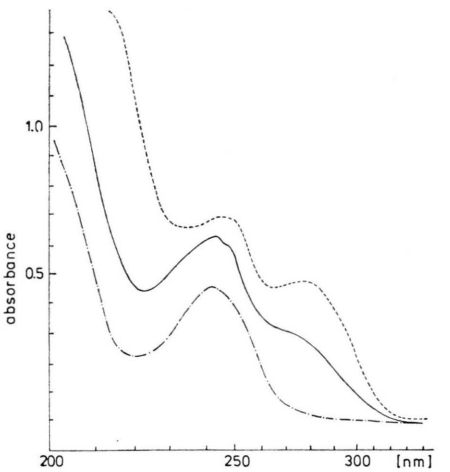


Fig. 5. Vapour phase ultraviolet absorption spectra, at temperatures indicated in brackets, of: ———, 9-ethylhypoxanthine (270 °C); — — —, 1,9-dimethylhypoxanthine (200 °C); — · — · —, 6-methoxy-9-methylpurine (320 °C).

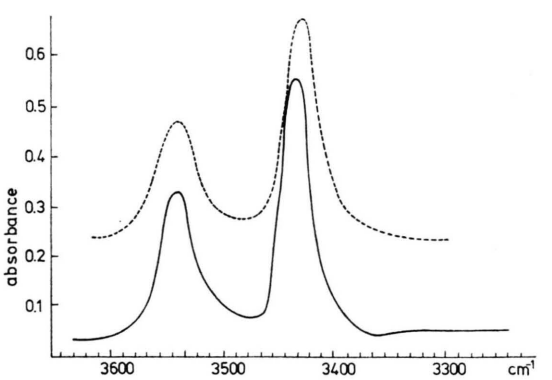
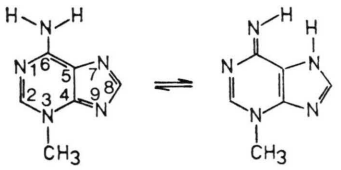


Fig. 6. Vapour phase infrared absorption spectra, at temperatures indicated in brackets, of: ———, 9-methyladenine (285 °C); — — —, 3-methyladenine (270 °C).

to the asymmetric and symmetric vibrations of the amino group. This is further supported by the fact that 3-methyladenine (Scheme 5), which has also



Va Vb

been established as being predominantly in the amino form **Va** in solution [19–21], displays the same two bands in the gas phase (Fig. 6, Table III), with relative integral intensities similar to those for 9-methyladenine. The analogous 3-benzyladenine, also shown to be in the amino form in $CDCl_3$ solution, likewise exhibits under these conditions two bands, at 3520 cm^{-1} and 3410 cm^{-1}, with relative integral intensities of 0.43 [21].

Table III. Vapour phase infrared symmetric and asymmetric NH_2 frequencies, ultraviolet absorption maxima, and heats of vaporization of N-methylated adenines.

Compound	Infrared bands [cm^{-1}]		Ultraviolet λ_{max} [nm]	ΔH_{vap} [kcal/mol]
	$\nu_{sym}(NH_2)$	$\nu_{asym}(NH_2)$		
9-Methyladenine	3438	3548	~ 200, 250	23 ± 2[a] 21 ± 2[b, c]
3-Methyladenine	3437	3548	~ 200, 250	22 ± 2[a] 18 ± 2[b]
N^6,9-Dimethyladenine	3465	—	—	—

[a] Calculated from $\nu_{sym}(NH_2)$.
[b] Calculated from $\nu_{asym}(NH_2)$.
[c] Clark *et al.* (ref. [9]) report a value of 26 kcal/mol.

Attempts to examine the vapour phase spectrum of the fixed imino form, 1,9-dimethyladenine, proved unsuccesful since, at the elevated temperature necessary to bring this derivative into the gas phase, it underwent rapid conversion to N^6, 9-dimethyladenine, presumably *via* the well-known Dimroth rearrangement [15]. However, 1,9-dimethyladenine in $CDCl_3$ solution displays a single band in the region of NH stretches, at 3300 cm^{-1}, assigned to the imino NH stretch [21]. The absence of any such band in this region in the vapour phase spectrum of 9-methyladenine (Fig. 6) provides additional evidence for its existence in the vapour phase as the amino form **IV a**.

Attempts were also made to examine 1-methyladenine in the gas phase, since it has been shown by several observers that this analogue is in the imino form in non-polar solvents. However the compound underwent thermal rearrangement to two major, and one minor, products, one of which was 6-methylaminopurine. The other two products have not been identified, but it should be noted that 6-methylaminopurine is the product of the Dimroth rearrangement of 1-methyladenine in alkaline aqueous medium. It is of interest, and also somewhat unusual, that both 1-methyladenine and 1,9-dimethyladenine undergo what appear to be Dimroth rearrangements in the vapour phase, *i. e.* in the apparent absence of water, which participates in this reaction [15].

From the temperature-dependent increase in intensities of $\nu_{sym}(NH_2)$ and $\nu_{asym}(NH_2)$, the heats of vaporization of 9-methyladenine and 3-methyladenine were calculated (Table III). Both compounds exhibited similar values, about 20 kcal/mol. Clark *et al.* [9] reported a value of 26 kcal/mol, based on the temperature-dependence on the UV spectrum, for 9-methyladenine, and a value of 29 kcal/mol from the temperature-dependence of the vapour pressure.

The vapour phase UV spectrum of 9-methyladenine (not shown) consists of two broad bands with maxima at about 200 nm and at 250 nm. Similar bands are displayed by 1,9-dimethyladenine and 3-methyladenine (Table III). In aqueous medium at neutral pH both bands of 9-methyladenine are red shifted by about 10 nm, While these data are consistent with 9-methyladenine being in the amino form in the gas phase, it should nonetheless again be emphasized that, in the absence of the IR data, this conclusion would not be fully convincing.

Concluding Remarks

The most striking conclusion from the results of this study is the fact that, for three natural nucleic acid bases, in the forms in which they occur in natural nucleic acids, the predominant tautomeric species are the same in the gas phase as in solution. Although attempts to examine 1-methylthymine (a formal analogue of thymidine) in the gas phase are still in progress, it may reasonably be assumed that its behaviour will not differ from that for 1-methyluracil.

The foregoing should be considered in relation to such non-natural analogues as 2-oxo- and 4-oxopyrimidines, which are predominantly in the keto forms in solution, whereas the tautomeric equilibrium constants in favour of the enol forms in the gas phase may be up to several orders of magnitude higher [4]. There are also a number of purines and pyrimidines, and their nucleosides, some of which are found naturally (but not as constituents of nucleic acids), and which are known to exist as a mixture of tautomeric forms in solution, e. g. 9-methylisoguanine and the naturally occurring nucleoside isoguanosine [3], 3-methylcytosine [22], isocytosine [23], 1-methyladenine (see ref. [21]), etc. It is perhaps no accident, therefore, that isoguanosine is not a normal base constituent of natural nucleic acids.

It is of additional interest that 1-methyl-5-fluorouracil (a formal analogue of 5-fluorouridine), which possesses a much more acidic proton on the ring N_3 than 1-methyluracil [24], and the base of which is readily incorporated into RNA in place of uracil, is also predominantly in the diketo form in the gas phase, as in solution [24]. The mutagenic activity of 5-fluorouracil has been variously ascribed to its greater tendency to adopt the 4-enol form, leading to mispairing with cytosine. The present findings do not provide support for such a concept.

The gas phase data on tautomerism of a number of heteroaromatic systems have been cited by Beak *et al.* [4, 7] as illustrative of the inadequacy of quantum chemical methods for evaluations of the predominant tautomeric species in a given system from the calculated differences in relative chemical binding energies of the protomeric isomers involved, and the obvious requirement for revision of the theoretical procedures currently applied to such problems. The fact that these same theoretical methods appear to give at least qualitatively correct results for 1-substituted uracil, and for 9-substituted hypoxanthine and adenine (since the vapour phase predominant tautomeric species for these are the same as for those in solution, which have formed the basis for checking the theoretically derived results), may consequently be entirely fortuitous. The experimental findings of this investigation should, in any event, prove of some significance in any reassessments of the validity of the theoretical procedures.

An additional point to be emphasized is that for those free, or 1- and 9-substituted, bases for which the predominant tautomeric species in the gas phase and solution are identical, attempts to interpret solvent effects on the electronic absorption spectra of these compounds, both experimentally and theoretically, should be considerably simpler of attainment.

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